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ORIGINAL ARTICLES

THE INCIDENCE OF STREPTOCOCCUS HEMOLYTICUS IN A RECENT EPIDEMIC OF INFLUENZA*

BY HOMER L. CONNER, MAJOR, M.C., U. S. A.

HISTORY

AT the U. S. A. General Hospital No. 19 there was made during the month of January, 1920, a nasopharyngeal survey of all the enlisted men on duty with the detachment. This survey was undertaken to find carriers of meningococci, pneumococci, streptococcus hemolyticus, B. diphtheriæ and B. influenzæ.

The survey was completed on the 29th, a total of 331 men having been examined. Just at this time when the survey was being completed, influenza began to be epidemic in Asheville. Quarantine was established at the hospital the 29th. Under its regulations nasopharyngeal cultures were taken from all classified as (a) all persons returning to the post after an absence of more than twenty-four hours; (b) all persons with respiratory disease that is acute in character; (c) all contacts to those with respiratory disease; (d) all cases suspected as possible influenza; (e) all convalescents from acute respiratory disease; (f) all attendants to patients in this hospital who might act as "carriers"; (g) all patients newly admitted to the hospital and all personnel newly joining the post.

In this period there were 822 individuals cultured a total of 2961 times.

All persons in the above classes were, in accordance with our regulations, cultured three times at intervals of forty-eight hours. Those showing streptococcus hemolyticus, pneumococcus, or B. influenzæ were quarantined until three consecutive negatives were obtained.

The number of cases detected, isolated, and diagnosed as influenza by the Medical Staff is small, being only forty-four. To this number the laboratory added twenty-seven who were carriers of the B. influenzæ.

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*From the Laboratories, U. S. A. General Hospital, No. 19, Oteen, N. C.

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Our first cases were among the T. B. patients, on two of whom a diagnosis of influenza was made the 29th of January. Between that time and February 12th, there were three more reported. It was among the detachment men that we had most of our cases, a total of thirty being reported. The first of these was admitted the tenth of February. Our last case was released from quarantine March 22.

We shall deal with the culturings of three periods. The preepidemic; the epidemic; the postepidemic.

The results are shown in detail in the tables which follow:

METHODS

All cultures have been taken from the posterior nasopharnyx by means of sterile swabs on copper wire which could be easily bent to the required angle at the time of taking the culture. These were introduced through the mouth, care being taken not to touch other than the nasopharnyx. With this swab blood plates were immediately streaked. We have used a beef infusion agar, P_H.7.6 as the foundation of all solid media used in this work.

This agar was kept in flasks, 200 c.c. to the flask, which was melted, and to which after the temperature of the agar had reached 90 C. was added one per cent of sterile defibrinated sheep blood. From this amount of agar twenty plates were poured, which were placed in the incubator overnight, after hardening, to test for sterility. Blood slants were made of this same mixture as needed. One plate of this "chocolate" agar was used for each person cultured, it being removed from the incubator at the time the culture was made, and after streaking with the swab it was returned immediately. After remaining in the incubator overnight at a temperature of 37° C. the plates were examined for the character of growth, smears made for Gram stain and morphology, and transfers made to Avery broth, blood and plain agar slants, or if necessary replated.

All steps and findings were carefully charted on forms prepared for that purpose.

Fished and replated colonies were incubated overnight at 37°C., then smeared and stained by Gram for morphology and purity, tested for bile solubility, and when indicated, agglutinated for type of pneumococcus according to standard methods.

Such Gram-negative bacilli as grew on blood agar and not on plain agar are classified as B. influenzæ.

TABLE I
CULTURES ON DETACHMENT MEN

	SMEAR					LOEFFLER					BLOOD PLATE							
Number of men cultured.	Spirilla	B. Fusiformis	Diphtheroids	Streptococci	Other	B. Diphtheriae	Diphtheroids	Streptococci	Staphylococci	Other	Meningococci	Strepto. Hemolyticus	Strepto. Viridans	Strepto. Indefinite	Staphylococci	Pneumococcus	B. Influenza	N. Catarrhalis	Other
331	85	18	4	298	48	1	12	178	314	97	0	20	5	185	225	95	5	2	99

PREEPIDEMIC PERIOD

Jan. 12 to Jan. 29, 1920.

During this period 331 men on duty with the detachment had cultures taken of the naso-pharynx. The results are summarized in Table I.

It should be stated that during this time there were no cases of acute respiratory disease among these men.

We therefore term the results of this survey the normal nasopharyngeal flora of these men.

EPIDEMIC PERIOD

Jan. 29 to Mar. 22, 1920

During this period there were nearly 3000 cultures taken which are classified in Tables II and III.

TABLE II

NASOPHARYNGEAL CULTURES—INFLUENZA EPIDEMIC

Date 1920	CASES AND SUSPECTS				CONTACTS				RETURN TO POST				RELEASE FROM QUARANTINE				ATTENDANTS			
	Total	Hemolytic Streptococcus	Pneumococcus	B. Influenzæ	Total	Hemolytic Streptococcus	Pneumococcus	B. Influenzæ	Total	Hemolytic Streptococcus	Pneumococcus	B. Influenzæ	Total	Hemolytic Streptococcus	Pneumococcus	B. Influenzæ	Total	Hemolytic Streptococcus	Pneumococcus	B. Influenzæ
Jan. 29 to Feb. 2	14	2	4	1	3	0	1	0	5	2	0	0	0	0	0	0	0	0	0	0
Feb. 2 to Feb. 6	29	3	1	0	6	0	0	0	30	0	1	1	54	1	3	0	70	15	4	0
Feb. 6 to Feb. 10	16	5	2	0	2	0	0	0	22	1	1	0	125	5	4	0	86	31	8	0
Feb. 10 to Feb. 14	25	8	3	1	4	2	0	0	25	3	1	0	94	11	3	0	0	0	0	0
Feb. 14 to Feb. 18	33	13	0	9	149	92	1	6	35	6	1	1	120	30	1	3	19	7	0	2
Feb. 18 to Feb. 22	18	11	0	2	109	24	0	0	42	10	0	0	148	51	0	8	46	9	0	0
Feb. 22 to Feb. 26	19	13	0	0	78	61	0	4	34	18	0	0	149	93	0	14	76	59	0	5
Feb. 26 to Mar. 1	24	12	0	0	73	47	0	3	42	25	0	1	173	25	0	1	72	47	0	3
Mar. 1 to Mar. 5	9	7	0	1	68	53	0	1	29	16	0	0	223	148	0	11	66	50	0	1
Mar. 5 to Mar. 9	4	3	0	1	48	22	0	1	2	0	0	0	112	69	0	1	48	26	0	1
Mar. 9 to Mar. 13	1	1	0	0	24	15	0	0	16	12	0	0	50	34	0	8	24	15	0	0
Mar. 13 to Mar. 17	0	0	0	0	10	2	0	0	0	0	0	0	36	14	0	4	10	2	0	0
Mar 17 to Mar. 22	5	2	0	0	12	2	0	0	0	0	0	0	37	5	0	1	12	2	0	0

It is to Table III that we especially call attention, showing as it does the progressive increase in percentage of Streptococcus hemolyticus carriers, which corresponds to the increase in number of cases of influenza detected and isolated. It shows also the decline in Streptococcus hemolyticus findings when the epidemic was on the wane, until in the postepidemic period, the number of such carriers again approaches what may be termed the normal percentage.

During this period, also, we made surveys of two complete wards, the whole nursing personnel, all detachment men on duty in the wards, the mess personnel, and the Emergency Hospital established in Asheville for the care of influenza.

The results of these surveys are given in Table IV.

Table IV also shows a seeming relationship of the streptococcus hemolyticus to influenza.

At the Emergency Hospital in Asheville, 90 per cent of the cases were carriers of this organism. The two wards were cultured, because it was from them that we were having cases reported that simulated a mild influenza like infection. The findings of so many carriers, both of the Streptococcus hemolyticus and B. influenzæ, is thought unusual. Subsequent culturings of these two wards, which

TABLE III

NASOPHARYNGEAL CULTURES

FLU PERIOD

DATE, 1920	NUMBER TAKEN	POSITIVE STREPTOCOCCUS HEMOLYTICUS	POSITIVE B. INFLUENZÆ	PER CENT STREPTOCOCCUS HEMOLYTICUS
Jan. 29 to Feb. 6	211	23	2	10
Feb. 6 to Feb. 10	251	44	0	17
Feb. 10 to Feb. 14	148	24	1	16
Feb. 14 to Feb. 18	356	160	21	44
Feb. 18 to Feb. 22	363	109	10	29
Feb. 22 to Feb. 26	356	244	23	68
Feb. 26 to Mar. 1	384	252	17	55
Mar. 1 to Mar. 5	395	274	14	69
Mar. 5 to Mar. 9	214	120	4	56
Mar. 9 to Mar. 13	115	77	8	66
Mar. 13 to Mar. 20	122	29	5	23
POST FLU PERIOD				
Mar. 22 to Mar. 26	48	13	0	27
Mar. 26 to Mar. 30	31	6	0	19
Mar. 30 to Apr. 6	43	10	0	23

TABLE IV

	NUMBER CULTURED	DATE	STREPTOCOCCUS HEMOLYTICUS	B. INFLUENZÆ	PER CENT STREPTOCOCCUS HEMOLYTICUS
Nurses	87	Feb. 6	49	0	56
Orderlies	67	Feb. 6	25	0	37
Mess Men	70	Feb. 7	10	0	14
Wards	79	Feb. 14	46	6	58
Emergency Hospitals	33	Feb. 17	30	12	90

were quarantined, and from which the B. influenzæ carriers were removed, gave a progressive decrease in positive Streptococcus hemolyticus findings, and there developed no other cases of the disease or carriers of the B. influenzæ.

The survey of the nurses, orderlies, and mess men, is valuable, as it shows the incidence of Streptococcus hemolyticus to vary according to the degree of contact with patients.

TABLE V

INCIDENCE OF STREPTOCOCCUS HEMOLYTICUS IN CLINICAL CASES OF "FLU" AND IN "CARRIERS." FOUR-DAY PERIODS

DATE	1/29 to 2/2				2/2 to 2/6				2/6 to 2/10				2/10 to 2/14				2/14 to 2/18				2/18 to 2/22			
	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS
CLINICAL CASES	0	0	0	0	0	0	0	0	2	0	0	0	0	2	8	0	2	0	10	0	3	0	8	0
POSITIVE STREPTOCOCCUS HEMOLYTICUS	2	0	0	0	0	0	0	0	2	0	0	0	0	2	8	0	2	0	10	0	3	0	8	0
POSITIVE B. INFLUENZÆ	1	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	5	0	3	0	3	0
	2/22 to 2/26				2/26 to 3/1				3/1 to 3/5				3/5 to 3/9											
CLINICAL CASES	1	0	2	0	1	0	1	0	0	0	0	0	0	0	1	0								
POSITIVE STREPTOCOCCUS HEMOLYTICUS	1	0	2	0	1	0	1	0	0	0	0	0	0	0	1	0								
POSITIVE B. INFLUENZÆ	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0								

		Str. Hem.	B. Influenzæ
Total Cases 44	T. B. Patients	12	11	4
	Nurses	2	2	2
	Detachment	30	30	13
	Civilians	0	0	0

B. INFLUENZÆ "CARRIERS"

DATE	1/29 to 2/2				2/2 to 2/6				2/6 to 2/10				2/10 to 2/14				2/14 to 2/18				2/18 to 2/22			
	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS	T. B. PATIENTS	NURSES	DETACHMENT	CIVILIANS
POSITIVE STREPTOCOCCUS HEMOLYTICUS	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	3	4	0	1	1	0	1	0
POSITIVE B. INFLUENZÆ	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	3	4	0	1	1	0	1	0
	2/22 to 2/26				2/26 to 3/1				3/1 to 3/5				3/5 to 3/9											
POSITIVE STREPTOCOCCUS HEMOLYTICUS	1	1	3	0	0	0	4	0	0	0	4	0	0	0	2	0								
POSITIVE B. INFLUENZÆ	1	1	3	0	0	0	4	0	0	0	4	0	0	0	2	0								

		Str. Hem.	B. Inf.
Total "Carriers" 27	T. B. Patients	6	6	6
	Nurses	6	6	6
	Detachment	14	14	14
	Civilians	1	1	1

Nurses who are in most intimate contact show the highest percentage; orderlies whose contact is not so great rank next, and mess men whose contact is the least show the lowest percentage.

That contact has its influence is also shown in Table II, attention being called especially to "Attendants." Here again we see the progressive increase in Streptococcus hemolyticus carriers which corresponds to the increase in the number of cases of influenza. It was possible by keeping a chart of daily cultures of the individual attendants to see them give on the first day or two of contact negative findings for the Streptococcus hemolyticus. After this first day or so this organism was in most a constant finding. After a period of exposure, varying from three to twenty days, we isolated in twelve of these attendants the B. influenzæ. They were isolated, but kept on duty in the "Flu" wards. None of them became ill.

The incidence of Streptococcus hemolyticus and B. influenzæ among the individual cases diagnosed as influenza and among carriers of B. influenza is shown in Table V.

It is to be noted that with one exception Streptococcus hemolyticus was always found in the nasopharyngeal cultures of those ill with the disease, and that carriers of B. influenzæ were also always carriers of this organism.

To state the case in another way, Table VI has been prepared to show the number of individuals cultured in the different periods, and of these, the number who never at any time had the Streptococcus hemolyticus isolated from the nasopharynx.

Here again is exactly the same picture as regards the low percentage of carriers of this organism in the preepidemic period; the steady progressive rise in the epidemic period; the approach to normal again in the postepidemic period.

TABLE VI

INDIVIDUALS CULTURED

PERIOD	DATE, 1920	NUMBER CULTURED	STREPTOCOCCUS HEMOLYTICUS	PER CENT
Preepidemic	Jan. 12 to Jan. 29	331	20	6
	Jan. 29 to Feb. 8	334	100	30
Epidemic	Feb. 8 to Feb. 20	313	201	65
	Feb. 20 to Mar. 20	175	155	89
Postepidemic	Mar. 22 to Apr. 13	122	29	24

POSTEPIDEMIC PERIOD

Mar. 22 to Apr. 13, 1920

During this period we took a series of nasopharyngeal cultures from various individuals as they came into the laboratory, for comparison.

The findings are charted in the tables above.

Also during this period a small series of experiments have been begun with about 100 cultures of Streptococcus hemolyticus isolated in the epidemic and postepidemic periods from different individuals.

TYPE OF STREPTOCOCCUS HEMOLYTICUS

Under this heading, and without going into detail about experiments, which are as yet not complete, we desire to record the most noteworthy characteristics of this organism, as it was observed during the epidemic.

Our observation of this organism shows, as has been noted by others, the variation in width and color of the hemolytic zone, to be governed largely by the circumstance under which it is isolated, and the medium on which it is grown.

We noted that in the preepidemic period hemolysis was in the vast majority of instances of the narrow zone type and of a marked greenish tint. Colonies of this type could not of surety be told from pneumococci, except by transplanting to broth and blood slant, which after incubating overnight were gram stained for morphology, tested for bile solubility, and agglutinated with pneumococcus sera. During this period we found and tested in this manner 120 cultures, 95 of them being classified as pneumococci because they were bile soluble.

After the first ten days of the epidemic period it became more and more unusual to find hemolysis of this type. The hemolytic zone was broader and had lost its greenish tint.

In the postepidemic period there was a return to the narrow type, and at the end of this period it is again usual.

During the epidemic and postepidemic periods over one hundred cultures of both broad and narrow zone hemolysis, which were isolated from as many different individuals, were tested on various sugars.

We found that, with two exceptions, all reduced lactose, dextrose, mannite, dextrin, and salicin. One culture did not reduce salicin; another did not reduce dextrose or dextrin.

These same cultures were transplanted to plain broth which was incubated overnight, and to which was then added 2 c.c. of a 5 per cent suspension of sheep blood corpuscles (in normal salt solution) to observe the length of time required to produce hemolysis. This varied from two to twenty-four hours.

TABLE VII

TABLE CLASSIFIED ACCORDING TO HOSPITAL PERSONNEL, SHOWING THE NUMBER OF INDIVIDUALS CULTURED AND PERCENTAGE OF POSITIVE STREPTOCOCCUS HEMOLYTICUS—EPIDEMIC PERIOD

1920	DETACHMENT MEN			T. B. PATIENTS			NURSES			CIVILIANS		
	NUMBER CULTURED	STREPTOCOCCUS HEMOLYTICUS	% STREP.	NUMBER CULTURED	STREPTOCOCCUS HEMOLYTICUS	% STREP.	NUMBER CULTURED	STREPTOCOCCUS HEMOLYTICUS	% STREP.	NUMBER CULTURED	STREPTOCOCCUS HEMOLYTICUS	% STREP.
Jan. 29, to Feb. 10	165	63	36	95	38	40	95	51	53	0	0	0
Feb. 10, to Mar. 22,	151	99	65	144	107	74	44	41	93	78	57	73
Total	316	162	51	289	145	49	139	92	66	78	57	73

The character of the hemolytic zone, as shown on the original blood plates, be it wide or narrow, has no influence as to the length of time which it takes to produce hemolysis under these conditions.

Our experiments point to the following conclusions:

1. That repeated transplants of colonies with narrow green tinted hemolysis on artificial media never produce wide zone hemolysis.

2. That wide zone hemolysis may, in some instances, persist for three, four, or more generations on blood plates; but when it once assumes the narrow green type it so remains on this medium.

3. That width of hemolytic zone is not dependent in the length of time which it takes to produce this, but rather on passage through man or animal.

4. That streptococci with zones of broad hemolysis are virulent; that narrow green tinted hemolysis is the quiescent stage.

DISCUSSION

The sequence of events which have made this paper possible are detailed in the above tables.

We see in the preepidemic period, which just antedated the discovery of cases of influenza in the hospital, the picture of the results of a nasopharyngeal survey which does not differ from that found by many other observers under similar conditions. It was among the men on whom this survey has been made that we eventually had the greatest number of influenza cases. We can therefore follow in their cultures the change in bacterial flora, especially as regards the Streptoccus hemolyticus, which was so constant and characteristic.

We are able also to make a like statement as regards our tuberculosis patients, on a large number of whom during the spring and summer of 1919 throat and sputum cultures had been made to determine the organisms which acted as secondary invaders. The results of this survey showed that the Streptococcus hemolyticus was to be found in this disease in a percentage which varied from thirty to sixty-six, the variation depending on the severity of the disease and the amount of lung involvement. Cases that were classified as the most severe being most often carriers of this organism, and then most often in its virulent form.

We have therefore two classes of individuals on whom we had, previous to the epidemic, made throat surveys and in whom the normal percentage of Streptococcus hemolyticus carriers was known.

In preparation for a possible epidemic of influenza the quarantine regulations which we recommended, provided that carriers of pneumococcus, Streptococcus hemolyticus, and B. influenzæ should be isolated. These provisions were carried out. Within ten days after the establishment of quarantine, however, it was found that so far as our cultural findings were concerned, we had not to deal with the pneumococcus as a factor in the epidemic.

The constantly increasing percentage of positive Streptococcus hemolyticus findings, which corresponded to the increase in number of cases of influenza diagnosed suspected, was evident early in the epidemic period.

What possible influence this section of the country, or to narrow the limits, this hospital, where so many cases of pulmonary tuberculosis, known carriers of the Streptococcus hemolyticus, had on the incidence of this organism in our local epidemic of influenza we cannot state. We know that persons in direct contact with active pulmonary tuberculosis are much more often than the normal individual not so exposed, carriers of this organism. This is well shown by our

surveys on nurses, orderlies, and mess men completed in the early days of the epidemic period.

In the order mentioned they are in most direct contact with known carriers of Streptococcus hemolyticus. Their cultural findings bear out the fact that contact has its influence.

That contact plays an important part in the spread of influenza we were able to show in the attendants on these cases whom we cultured every day. The first culture on assignment to a "Flu" ward would be negative for organisms which we associated with influenza, but within a day or two all commenced to show the Streptococcus hemolyticus, and in twelve, after periods varying from three to twenty days, we isolated the B. influenzæ.

The findings of Streptococcus hemolyticus were always antecedent to those of B. influenzæ. This fact brings forth a desire to speculate as to which organism is the primary infecting agent in influenza, but as this subject is outside the scope of this paper, we leave it for others to discuss, theorize on, or prove.

The results of cultures taken from individuals other than those already mentioned, classified according to status, i. e., civilian employees, duty personnel returning from leave or newly joining the Post, newly admitted patients, and visitors—show a similar increase in incidence of Streptococcus hemolyticus during the epidemic period.

We therefore assert that all individuals connected with this hospital, no matter what their status, or how great the likelihood of being chronic carriers of Streptococcus hemolyticus through contact with, or in fact being a tuberculosis patient, show in the epidemic period an increase in this carrier state. The normal for even those with the most advanced forms of pulmonary tuberculosis we have found never to be more than 66 per cent. In this epidemic we had not to deal with this class of individual. The tubercular patients who developed influenza and those exposed to the disease through contact were among those that we term ambulatory. In them our previous studies gave only twenty to thirty per cent as carriers of Streptococcus hemolyticus.

Our tables show that with one exception all cases of influenza, and all carriers of the influenza bacillus were also carriers of Streptococcus hemolyticus.

The tables which deal with, and classify the separate individuals cultured, show a percentage of positive Streptococcus hemolyticus findings, which at the height of the curve corresponds almost exactly to the findings which we obtained from cultures taken for comparison from the entire personnel of the Emergency Hospital in Asheville.

We have completed this study by showing in the postepidemic period the return to what approached, in this institution, the normal percentage of Streptococcus hemolyticus carriers.

CONCLUSIONS

1. That there was, in this locality, a direct relationship between the streptococcus hemolyticus and the influenza epidemic.

CHEMICAL CHANGES IN THE BLOOD IN DISEASE*

VI. Cholesterol

By Victor C. Myers, Ph.D., New York City

DURING the past ten years cholesterol has been the subject of many varied and extended investigations, the larger number of which have been carried out on the blood. Although many of these studies have furnished information of great value regarding the physiologic and pathologic role of this interesting lipoid, they have not disclosed many deviations which can be utilized to advantage in diagnosis.

The importance of cholesterol is indicated by its widespread occurrence in the animal body. Cholesterol is obviously, therefore, a constituent of our various animal foods, from which probably much of the cholesterol of the body is derived. According to Fraser and Gardner,[1] the phytosterols of the plant foods are transformed to cholesterol in the body, evidently furnishing a portion of our supply of this substance. Whether or not cholesterol is synthesized in the body remains a disputed question. Luden[2] has clearly demonstrated the augmenting influence of animal foods, particularly eggs, butter and meat on the blood cholesterol, while Rothschild and Rosenthal[3] have advocated the use of diets low in cholesterol in the treatment of certain types of cholelithiasis with hypercholesterolemia.

Although cholesterol is a monatomic, simple, unsaturated, secondary alcohol, and further possesses the character of a complicated terpene, its close association with fat and lecithin, and the fact that it may combine with a fatty acid to form a fat makes it of special interest in this connection. Bloor,[4] in particular, has given us much useful information regarding the variations in these three lipoids in the blood. Normally the "total fat" content of the blood plasma amounts to 0.6 to 0.7 per cent, but in the severe lipemia of diabetes figures as high as 26 per cent have been observed. The cholesterol content of the blood runs fairly parallel with the total fatty acids in all cases, including lipemia, and on this account is an excellent index of the degree of lipemia in diabetes. Cholesterol occurs in the blood in both the free and combined state. Free cholesterol is present in the corpuscles and to some extent in the plasma, and the cholesterol esters in the plasma alone. Bloor and Knudson[5] have found that in whole blood the average percentage of cholesterol in combination as esters is about 33.5 per cent, and in the plasma 58 per cent of the total cholesterol. Most of the data recorded in the literature, however, are for the total cholesterol of the blood, some of the results being on the plasma or serum, others on the whole blood. Normally, the concentration of cholesterol is nearly the same in the plasma and the whole blood, although, if anything, the plasma content is slightly higher, and patho-

*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York City.

logically it seems to be subject to somewhat greater variations. That such is the case is well illustrated in the following Table I abbreviated from Grigaut.[6]

TABLE I

DISTRIBUTION OF CHOLESTEROL IN THE BLOOD*

CONDITION	CHOLESTEROL IN PER CENT			
	SERUM	PLASMA	WHOLE BLOOD	CORPUSCLES
1. Normal man	0.168	0.168	0.159	0.141
2. Normal man	0.170	0.170	0.150	0.130
3. Normal woman	0.174	0.170	0.168	0.171
4. Normal woman	0.175	0.175	0.165	0.140
5. Carcinoma of the pancreas with jaundice	0.071	0.068	0.105	0.110
6. Pneumonia	0.098	0.098	0.110	0.150
7. Carcinoma of the liver with jaundice	0.222	0.228	0.198	0.170
8. Diabetes	0.246	0.246	0.201	0.137
9. Cholelithiasis	0.276	0.270	0.225	0.180
10. Nephritis	0.450	0.450	0.285	0.150
11. Nephritis	0.514	0.514	0.264	0.135
12. Carcinoma of the pancreas with jaundice	0.840	0.840	0.540	0.195

*Taken from Grigaut.[6]

TABLE II

THE CHOLESTEROL CONTENT OF THE BLOOD IN VARIOUS PATHOLOGICAL CONDITIONS

CASE	AGE	SEX	CHOLESTEROL	CO_2 COMBINING POWER	SUGAR	UREA N	CONDITION
			per cent	c.c. to 100	per cent	mg. to 100 c.c.	
1. H.B.	20	♂	0.82	21	0.39		Diabetes, Case 1 showing a total fat content of 7.1 per cent.
2. A.F.	20	♂	0.63	31	0.86		
			0.57	38	0.41		
3. U.W.	49	♂	0.20		0.79		
			0.25	12	1.16		
4. H.R.		♂	0.34	35	0.30		
5. W.A.	45	♂	0.11	50	0.25		
6. W.F.	25	♂	0.34			100	Bichloride poisoning
7. E.E.	30	♀	0.28			33	Nephritis
8. E.B.	24	♂	0.28				
9. I.D.	17	♀	0.22			170	
10. J.W.	34	♂	0.16			63	
11. M.H.	71	♀	0.23				Arteriosclerosis
12. L.W.	62	♀	0.21				
13. D.G.	23	♀	0.24				Moderately severe pellagra
14. H.C.	44	♀	0.13				Cholelithiasis, confirmed by operation, no jaundice
			0.16				
15. W.L.	15	♀	0.18				
			0.21				
16. F.T.	21	♂	0.21				Catarrhal jaundice
17. R.K.	51	♂	0.29				Obstructive jaundice
18. H.H.	47	♂	0.20				Carcinoma of stomach, early
19. M.L.	45	♂	0.12				Carcinoma of stomach, late
20. L.M.	34	♀	0.23				Pregnancy, 8 mos., eclampsia
21. O.G.	48	♂	0.07				Pernicious anemia, plasma 0.065%, washed cells, 0.12% plasma, 0.065%, washed cells, 0.13%
22. F.S.	50	♂	0.07				

Chauffard, Laroche and Grigaut[7] have given 0.15 to 0.18 per cent as the normal value for the cholesterol content of blood serum, figures which closely agree with the findings of subsequent workers. This would make the figures for whole blood about 0.14 to 0.17 per cent. Pathologically, many conditions have been recorded in which a hypercholesterolemia was found, while in a few conditions hypocholesterolemia has been noted. In general it may be stated that hypercholesterolemia is found in arteriosclerosis, nephritis, diabetes (especially with acidosis), obstructive jaundice, in many cases of cholelithiasis, in certain skin diseases, in the early stages of malignant tumors, and in pregnancy. The chief condition in which low values for cholesterol are found is anemia.

Figures for the cholesterol content of whole blood, which are intended to be representative of the findings in the various pathologic conditions mentioned above, are given in Table II. The figures are taken partly from Gorham and Myers[8] and partly from some of our more recent observations. Grigaut[6] has presented a most excellent discussion of the cholesterol content of blood, while the papers of Bloor[4] (includes data on other lipoids), Denis,[9] and Gorham and Myers[8] all review this general subject, including the literature.

Rothschild and Wilensky[10] give an excellent outline of the factors influencing the blood cholesterol:

1. The cholesterol content of the blood is lowered:
   (a) By a diet which is poor in lipoids.
   (b) By the occurrence of high temperatures.
2. The cholesterol content of the blood is increased:
   (a) By a diet excessively rich in lipoids.
   (b) By the presence of other diseased conditions, especially diabetes, arteriosclerosis, and nephritis.
   During pregnancy. This lasts for a variable period after evacuation of the uterus.
   (d) By the presence of obstruction in the common bile duct. If the obstruction, however, it not absolute, as indicated by the degree of accompanying jaundice, the cholesterol content of the blood may not be increased.

Although the observations recorded in Table II are given only on whole blood, with the exception of the cases of anemia, it is apparent from the data of Table I that greater variations take place in the cholesterol of the serum or plasma. Nevertheless a significant change in the cholesterol is readily evident from observations made on whole blood.

A more marked hypercholesterolemia may be found in the lipemia of diabetes than in any other condition. The lipemia of the first two cases of Table II, showing cholesterol figures of 0.6 to 0.8 per cent, was very marked. Although the ordinary case of diabetes at the present time does not show lipemia in the sense that the blood is milky, still the lipoids of the blood are increased in all types of the disease. Joslin, Bloor and Gray[11] found that the average quantity of lipoids in the whole blood with the Bloor method amounted to 0.59 per cent in 19 normal individuals, but was increased to 0.83 per cent in 30 mild diabetics, to 0.91 per cent in 37 moderately severe diabetics, and to 1.41 per cent in 55 severe cases of diabetes. This holds for all three groups of lipoids. They state, "The increase in cholesterol is significant and suggestive, and seems indeed

pathognomonic of the prolonged diabetic hyperlipemia, since Bloor has found it lacking in the acute lipemia of overfeeding which is characterized by an increase in the total fatty acid alone." On this account the determination of the cholesterol alone should give valuable information regarding the lipoid content of the blood in diabetes.

Although many observers have noted and studied the hypercholesterolemia of nephritis, it is not possible as yet to give a satisfactory interpretation of these findings. In the case of arteriosclerosis, however, it is worthy of note that histologic changes have been observed in the aorta after the experimental administration of cholesterol.

Since gallstones are largely composed of cholesterol, it is reasonable to suppose that their appearance might be associated with an increase in the cholesterol content of the blood. Henes[12] has maintained that this is the fundamental and primary factor in the formation of gallstones. Although it seems quite probable that a hypercholesterolemia is presented during the early period of the formation of the calculi, analytical data show wide variations in the blood cholesterol,[3, 8, 10] the findings ranging from low normals to figures that are definitely increased. Rothschild and Rosenthal[3] emphasize the fact that in a certain group of cases the hypercholesterolemia is very persistent and operation affords only temporary relief. They believe that properly selected low cholesterol diets have been very helpful in these cases.

It is logical to expect that in obstructive jaundice the cholesterol content of the blood should be elevated and bear a fairly definite relation to the intensity of the icterus. Rothschild and Felsen[13] have shown, however, that in conditions associated with hepatic disorders the cholesterol of the blood is not increased, but usually reduced, while in so-called hemolytic icterus, there is no increase of blood cholesterol.

It would appear that in the early stages of malignancy, the blood cholesterol was somewhat elevated or normal, while in the late stages the figures are below normal. Luden[2, 14] has called attention to the fact that a diet which increases the blood cholesterol coincidently weakens the lymphoid defense. She suggests that in persons predisposed to carcinoma an increase of the cholesterol and a weakening of the lymphoid defense, such as may occur with the prolonged use of a high cholesterol diet, may perhaps result in the development of carcinoma.

The hypercholesterolemia of pregnancy is well known. Chauffard, Laroche and Grigaut[15] found that the increase begins about the fourth month of pregnancy and becomes progressively greater as full term is approached. Slemons and Curtis[16] have made the interesting observation that cholesterol esters are frequently absent from fetal blood. In this case the cholesterol is exclusively in the free form, and furthermore, the free cholesterol of both maternal and fetal blood are identical. Apparently the normal placental partition is permeable for free cholesterol but impermeable for cholesterol esters.

That the cholesterol of the blood (plasma) is lowered in anemia has been recognized for some time. When the antihemolytic action of cholesterol is recalled, it will be seen that this observation may possess some practical significance. The therapeutic administration of cholesterol in this condition has received attention from Italian investigators and has apparently been followed by

beneficial results. Pacini[17] (in this country) has recently presented some interesting observations on the blood cholesterol in pernicious anemia, giving data on the whole blood, serum and cells. He found the cholesterol markedly decreased in the serum but relatively increased in the cells. He administered cholesterol in the form of lanolin as an inunction, and believed that he obtained definite benefit.

Estimation of Cholesterol

Cholesterol was one of the first constituents of the blood to be determined colorimetrically. Several years before the development of the colorimetric methods of blood analysis described in the earlier papers of this series, Grigaut[6, 18] had already described (1910) a colorimetric procedure of estimating the cholesterol content of blood. In the development of the color Grigaut made use of the Liebermann-Burchard reaction, and the technic of this part of the test is still carried out essentially as he originally described it. Two years after the publication of Grigaut's method, Weston[19] described a procedure in which the Salkowski color reaction was employed. The Liebermann-Burchard reaction is better suited, however, for use in this connection. In 1913 Autenrieth and Funk[20] described a slight modification of the Grigaut technic and adapted it to use with the Autenrieth-Königsberger (Hellige) colorimeter. This modification has been extensively employed and many references may be found to work carried out with the Autenrieth-Funk method. It would seem only fair to Grigaut, however, that this method should bear his name. A number of different workers have described procedures of cholesterol extraction upon which the colorimetric method of Grigaut is applied.

In the case of the excellent but laborious gravimetric digitonin method of Windaus[21] for the estimation of total cholesterol, saponification of the cholesterol esters is necessary, since only the free cholesterol is precipitated by the digitonin. Cholesterol esters give the color reaction as well as does the free cholesterol. This fact does not appear to have been recognized until recently, since the directions for the colorimetric estimation have almost invariably called for a preliminary saponification. As pointed out by Bloor,[22] this saponification is unnecessary and the colorimetric estimation of the cholesterol thus becomes further simplified.

Bloor has suggested a method of extraction[23] for the cholesterol (and other lipoids) which is very simple and would appear to be complete, but the results obtained with the method as finally carried out (second method) are higher than those by the older methods, and rather irregular, owing apparently, to the presence in the extracts used of substances interfering with the Liebermann-Burchard color reaction for cholesterol. These high results have been criticized by Mueller[24] and Weston,[25] who are of the opinion that they are due to the admixture of brownish tints frequently obtained in the final development of the color. Luden[26] obtained similar high findings with Bloor's second method, but believed that these resulted from a combination of bile pigments and bile acids. With Bloor's first method the alcoholic ether extract was saponified with sodium ethylate, this procedure being omitted with the second method. Luden's data bearing

on this point are very interesting. Our observations on this subject are in harmony with her conclusions.

Myers and Wardell[27] have described a comparatively simple method of direct cholesterol extraction which appears to yield reliable results. At any rate added cholesterol may be quantitatively recovered, and good checks obtained with the totally different Windaus method. With this method 1 c.c. of blood is mixed with plaster of Paris and dried. In addition to putting the blood into a

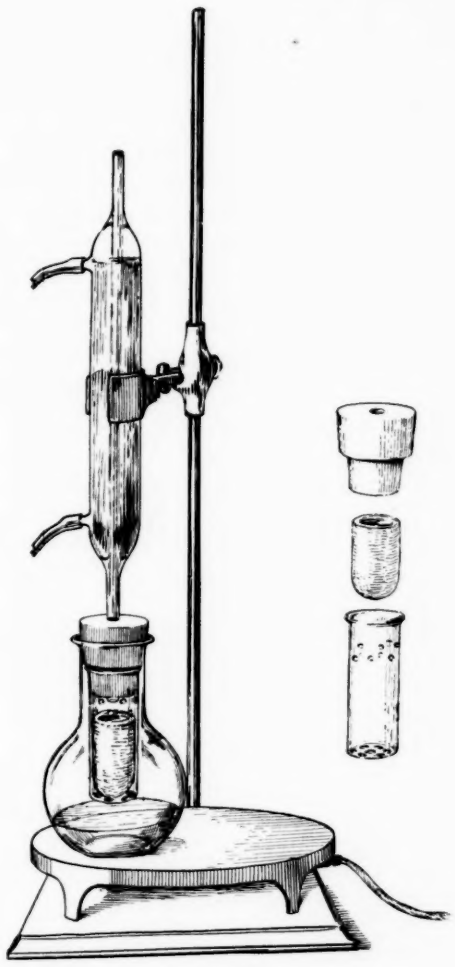

Fig. 1.

finely divided and readily extractable condition, this calcium salt apparently holds back substances which add to the color development with the Bloor technic. After reaching its greatest intensity the cholesterol color fades rather rapidly and for this reason Myers and Gorham[28] suggested the use of naphthol green B as a standard. This dye excellently matches the cholesterol color and appears to be permanent.

In carrying out the color reaction it is essential that the reagents should be anhydrous. Poor acetic anhydride will give a weak color development. For

this reason it is desirable before developing a series of unknown solutions to first check the quality of reagents by developing a solution of pure cholesterol.

Method.[27]—For the determination, 1 c.c. of blood, plasma or serum is pipetted into a porcelain crucible or small beaker containing 4 to 5 gm. of plaster of Paris, stirred, and dried, preferably in a drying oven for an hour. It is now emptied into a small paper extraction shell (4 cm. long) and then inserted in a short glass tube* (2.5x7 cm.) in the bottom and sides of which are a number of small holes (Fig. 1). This is now attached to a large cork on a small reflux condenser and the tube and cork inserted in the neck of a 150 c.c. extraction flask containing about 20 to 25 c.c. of chloroform. (We have frequently run 3 to 6 extractions simultaneously on the same hot plate.) Extraction is continued for 30 min., on an electric hot plate, the chloroform made up to some suitable volume, such as 20 c.c., filtered if necessary, and colorimetric estimation carried out as follows: 5 c.c. of the chloroform extract are pipetted into a dry test tube, and 2 c.c. of acetic anhydride and 0.1. c.c. of concentrated sulphuric acid (best with 0.1 c.c. pipette) are added. After thorough mixing, the solution is placed in the dark for exactly 10 min.** to allow the color to develop, and then compared with a standardized 0.005 per cent aqueous solution of naphthol green B in a Bock-Benedict or Kober colorimeter. If the Duboscq colorimeter is used, it is necessary that the cups should be remounted in plaster of Paris, instead of balsam.

With a good grade of acetic anhydride, it has been found that when an 0.005 per cent solution of naphthol green B is used as a standard and set at 15.5 mm. on the Duboscq or Kober instrument, 0.4 mg. of cholesterol in 5 c.c. of chloroform treated with 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid will read 15 mm. The color curve for both the cholesterol and naphthol green B appear to fall in a straight line so that readings somewhat above or below the standard are accurate.

If a cholesterol standard containing 0.4 mg. to 5 c.c., or a naphthol green B standard of equivalent strength, are employed, the following formula may be used for the calculation $\frac{S}{R} \times 0.0004 \times \frac{D}{5} \times 100 =$ cholesterol content of blood in per cent, in which S stands for the depth of standard in mm., R for the reading of the unknown, 0.0004 the equivalent amount of cholesterol in 5 c.c. of chloroform, D the dilution of the chloroform extract from the 1 c.c. of blood, 5 the dilution of the standard and 100 the factor for 100 c.c. For example $\frac{15}{15} \times 0.0004 \times \frac{20}{5} \times 100 = 0.160$ per cent.

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*Originally we made these tubes from large test tubes by drawing out the tubes, blowing new bottoms (round) and then punching a number of holes with a white hot, malleable iron wire. Later we tried alundum thimbles, in which two small holes had been drilled near the top to easily permit the entrance of the chloroform vapor. Although this obviated the necessity of paper extraction shells, cups could not be found that were sufficiently porous to make a rapid extraction possible. The flat bottom tubes illustrated are an advantage in that they will stand, and the thimble cannot be sucked tight against the bottom of the tube.

**In order to get the proper temperature for color development in warm weather it is advisable either to keep the reagents in a cool place or to insert the tubes in water during the development of the color.

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THE VALUE OF THE COMPLEMENT-FIXATION TEST IN TUBERCULOSIS*

PRELIMINARY PAPER

(With Observations, Using the Hecht-Weinberg Modification)

BY ROY UPHAM, M.D., F.A.C.S., AND A. J. BLAIVAS, BROOKLYN, N. Y.

IT is a well-known fact that the antigen is the most important factor in complement-fixation work. The antigen of choice is the one that fixes complement most often in a given disease. It is the object of the writers to determine, first, which is the most delicate antigen, and second, to interpret the laboratory findings.

All cases here reported were patients that consulted one of us (Upham) for gastrointestinal disturbances and tuberculosis was not suspected. Inasmuch as a Wassermann test was made on each patient as a routine, it was thought advisable to include the complement-fixation test for tuberculosis, using the antigens of Petroff, Miller, and Fleisher-Ives. This will, of course, account for the large percentage of negatives.

METHODS EMPLOYED

The Wassermann reaction was made on each patient using both cholesterin and ether-soluble, acetone-insoluble antigens. As stated above, in the complement-fixation test for tuberculosis, the antigens of Petroff, Miller and Fleisher-Ives were used. In the complement fixation tests the results were estimated in terms of +, ++, +++ and ++++ but any result below +++ was considered negative. In the Hecht-Weinberg modification, any result below ++++ was considered negative.

The work was done separately, charted, and the laboratory had no information whatsoever regarding the patient. The temperature, weight, physical findings and x-ray examinations were made on each patient. Each patient also received from 0.5 to 2.5 milligrams of old tuberculin and the von Pirquet test.

The tables show that we have classified our cases as follows:

1. As they presented themselves.
2. Clinical tuberculosis.
3. Tuberculosis suspects.
4. Nontuberculous.
5. Other diagnoses.

Ives and Singer[1] claim that the complement-fixation test recognizes only active tuberculosis. In Table I it will be observed that cases 20 and 45 were arrested cases and still gave strong positive fixation tests. We found some cases

*From the Laboratories of the Prospect Heights Hospital, Brooklyn, N. Y.

TABLE I

CASE NO.	NAME	COMPLEMENT-FIXATION TEST FOR TUBERCULOSIS: PETROFF ANTIGEN	COMPLEMENT-FIXATION TEST FOR TUBERCULOSIS: MILLER ANTIGEN	COMPLEMENT-FIXATION TEST FOR TUBERCULOSIS: FLEISHER-IVES ANTIGEN	MODIFICATION OF HECHT FOR TUBERCULOSIS: PETROFF ANTIGEN	MODIFICATION OF HECHT FOR TUBERCULOSIS: MILLER ANTIGEN	MODIFICATION OF HECHT FOR TUBERCULOSIS: FLEISHER-IVES ANTIGEN	H. I.*	WASSERMANN	H. W. G.**	DIAGNOSIS
1.	J.A.B.	–	–	–	–	–	–	4	–	–	Intestinal intoxication
2.	H.M.	–	–	–	–	–	–	2	–	++	Ulcer, Asthma syphilis (?)
3.	C.B.M.	+	+	+	++	++	++	2	–	–	Intestinal intoxication
4.	I.F.W.	–	–	–	±	±	±	4	–		Gastrocoloptosis
5.	A.R.	–	–	–	NOT	MADE	NOT	MADE	NOT	MADE	Cancer of stomach
6.	G.W.H	–	–	–	NOT	MADE	NOT	MADE	NOT	MADE	Intestinal intoxication, Nephritis
7.	J.L.M.	–	–	–	–	–	–	8	–		Gastrocoloptosis, Tuberculosis (?)
8.	E.R.G.	++	++	++	++++	++++	–	6	–	±	Tuberculosis, mucous colitis
9.	W.C.	–	–	–	+	–	–	2	–	++	Gastrocoloptosis, Syphilis (?)
10.	G.A.M.	–	–	–	–	–	–	5	–	–	Cholelithiasis
11.	C.B.G.	–	–	–	++	+	+	4	–	–	Tuberculosis (?)
12.	P.B.L.	±	±	±	+	–	+	7	–	–	Subacid Gastric Catarrh
13.	C.G.	–	–	–	–	–	–	6	–	–	Gastrocoloptosis, Tuberculosis (?)
14.	G.F.	–	–	–	++	–	+	7	–	–	Intestinal Toxemia
15.	S.P.G.	++	++	++	++	++	++	4	–	+	Intestinal Toxemia, Nephritis
16.	G.V.W	++++	–	–	+++	+++	+++	9	–	–	Gastric catarrh, Tuberculosis
17.	A.P.	–	–	–	–	–	–	9	–	–	Cholelithiasis
18.	M.H.	++	–	–	++++	++	–	10	–	–	Gastrocoloptosis, Tuberculosis
19.	H.R.W	+++	–	++	++++	++++	++++	9	–	++++	Cancer
20.	R.T.S.	+++	++++	++	++++	++++	++++	9	–	–	Arrested Tuberculosis, Gastric catarrh
21.	J.O.	+++	++++	+++	++++	++++	++++	9	–	–	Gastrocoloptosis, Tuberculosis
22.	R.R.	+	+	+	++	++	++	3	–	–	Gastric Catarrh, Tuberculosis
23.	W.J.F.	–	–	–	–	–	–	2	–	–	Cholecystitis
24.	C.C.B.	–	–	–	++	++	++	3	–	–	Intestinal Toxemia, Nephritis
25.	J.K.	–	–	–	–	–	–	2	–	–	Gastrocoloptosis
26.	A.S.B.	+	+	+	++++	++++	++++	3	–	–	Intestinal Toxemia
27.	H.S.	–	–	–	–	–	–	8	–	–	Anemia, Tuberculosis (?)
28.	C.H.B.	–	–	–	–	–	–	4	–	–	Anemia, Tuberculosis (?) Gastric Catarrh
29.	A.G.B.	–	–	–	–	–	–	9	–	++	Gastrocoloptosis
30.	E.H.	–	–	–	–	–	–	4	–	–	Gastrocoloptosis
31.	M.J.N.	–	–	–	+	++	–	4	–	–	Intestinal Toxemia, Anemia
32.	A.T.	–	–	–	–	–	–	5	–	–	Intestinal Toxemia
33.	J.V.L.	–	–	–	–	–	–	3	–	–	Hemorrhoids, Gastric Catarrh
34.	G.G.	–	–	–	–	–	–	5	–	–	Intestinal Toxemia
35.	G.R.	–	–	–	–	–	–	3	–	–	Gastrocoloptosis, Tuberculosis (?)
36.	P.G.	–	–	–	–	–	–	8	–	–	Gastrocoloptosis
37.	G.P.B.	++	++	++	++++	++++	++++	4	–	–	Intestinal Toxemia
38.	A.M.L.	++++	++++	++++	++++	++++	++++	6	–	–	Gastric catarrh, Tuberculosis (?)
39.	T.M.C.	–	–	–		–	–	7	–	–	Gastrocoloptosis
40.	M.M.G.	–	–	–	++++	++++	++++	6	–	–	Intestinal Toxemia, Nephritis
41.	R.S.D.	–	–	–	–	–	–	10	–	–	Gastrocoloptosis, Tuberculosis (?)
42.	J.F.C.	–	–	–	++++	++++	++++	3	–	++	Cholecystitis, Syphilis
43.	J.C.M.	–	–	–	NOT	MADE	NOT	MADE	–	NOT MADE	Terminal Gastric Catarrh
44.	B.	++++	–	–	++++	++++	–	7	–	–	Terminal Gastric Catarrh
45.	R.M.W	++++	–	–	++++	–	++++	8	–	–	Ulcer, Arrested Tuberculosis
46.	G.E.H.	–	–	–	NOT	MADE	NOT	MADE	–	NOT MADE	Intestinal Toxemia, Anemia
47.	M.B.H.	–	–	–	–	–	–	7	–	–	Gastrocoloptosis, Tuberculosis
48.	C.E.B.	–	–	–	–	–	–	9	–	–	Gastrocoloptosis
49.	E.W.C.	–	–	–	–	–	–	9	–	–	Intestinal Toxemia
50.	N.M.	–	–	–	–	–	–	8	–	–	Intestinal Toxemia, Tuberculosis (?)
51.	M.W.	–	–	–	–	–	–	6	–	–	Intestinal Toxemia, Nephritis
52.	F.E.I.	–	–	–	–	–	–	10	–	–	Intestinal Toxemia
53.	M.K.	–	–	–	–	–	–	9	–	–	Amebic Dysentery
54.	C.S.	++++	++++	++++	NOT	MADE	NOT	MADE	–	NOT MADE	Gastrocoloptosis, Chronic appendix
55.	L.A.	++	+	++	++++	++	++++	3	–	++++	Gastrocoloptosis
56.	C.R.	–	–	–	–	–	–	2	–	–	Tuberculosis
57.	M.G.	–	–	–	++	++	+	4	–	–	Syphilis and Tuberculosis
58.	T.J.O.	–	–	–	–	–	–	5	–	–	Tuberculosis
59.	D.	–	–	–	–	–	–	10	++++	++++	Syphilis
60.	Dr.M.	–	–	–	–	–	–	1	–	–	Intestinal Toxemia

*Hemolytic Index.

**Hecht-Weinberg Test.

that were clinically tuberculous gave negative reactions and it was these results that led us to try the principle of Hecht.

While heating serum to 56° C. (inactivating) destroys any natural complement that may be present, it does not, however, destroy the natural amboceptor that may be present. Inactivating serum in the Wassermann test may destroy some of the syphilitic antibodies present and thus also render the test more liable to error. Heating serum in the complement-fixation test for tuberculosis may therefore also destroy some of the tuberculous antibodies present in the serum. This is only a theory of the writers and has not as yet been confirmed. The fact that in the original technic the amboceptor is not destroyed, and a titrated amount of amboceptor is added to the serum regardless of the amount of natural amboceptor present, proves that an excess of amboceptor is added to the serum. This may, of course, render a negative reaction when in reality it should be positive. Bearing this fact in mind we performed the Hecht-Weinberg modification in the complement-fixation test for tuberculosis.

LABORATORY TECHNIC

The blood was collected in the usual manner and allowed to clot. It was then centrifugalized and the serum was drawn off using a sterile pipette. The bloods were examined on the day that they were collected.

Antigens.—The antigens used in this series were:

1. Petroff's glycerine antigen which was furnished us by Petroff.
2. Miller's antigen which was generously given us by Dr. M. Kahn of Beth Israel Hospital, New York.
3. Fleisher-Ives antigen which was furnished us by Dr. G. Ives.

Complement.—Pritchard and Roderich[2] observed that guinea pigs gave a strong positive reaction and upon investigation found that the sera of the guinea pigs were at fault. The writers made complement-fixation tests on all guinea pigs and can therefore confirm the observation of Pritchard and Roderich. We then discarded those pigs that gave a positive complement-fixation for tuberculosis and used only those that gave clear-cut negative reactions.

The guinea pigs were bled from the heart and the sera of four or five guinea pigs were pooled. The complement was then diluted 1-10 using normal saline as a diluent.

Amboceptor.—The rabbit antisheep amboceptor was prepared and titrated in the usual way. Two units were used throughout the tests.

Sheep Cells.—The blood was obtained from the jugular vein into a wide-mouth bottle containing some sterile glass beads. It was then thoroughly shaken until completely defibrinated. The blood was then centrifugalized at about 3000 revolutions per minute about six or seven times. After the last time the supernatant fluid was tested for albumin using the nitric acid contact test. If there were any traces of albumin, it was centrifugalized again three times and the albumin test was repeated. A five per cent suspension of sheep cells in normal saline was used throughout the serological work.

Salt Solution.—In this work, 0.85 per cent salt solution was used. Care was taken that it was always sterile.

Glassware.—All glassware was chemically cleaned and sterilized. Although it is not absolutely necessary to have the glassware sterile, it was thought advisable to have it so throughout the serologic work.

Complement-Fixation Technic.—One unit of Complement, two units of amboceptor and one unit of the antigens were used. One c.c. of a 5 per cent suspension of sheep cells was used. After adding 0.2 c.c. of serum, then the saline, complement and the antigens, the tubes were placed in the water-bath for one hour at 37.5° C. After this time, the amboceptor and sheep corpuscles were added, the tubes were again incubated for one hour and then removed and read.

Technic for the Modification.[3]—Place 0.1 c.c. of the unheated serum into each of the twenty small test tubes which are placed in a special rack. Put 1 c.c. of the normal saline in Tube 1; 0.9 c.c. in Tube 2; 0.8 c.c. in Tube 3; 0.7 c.c. in Tube 4; 0.6 c.c. in Tube 5; 0.5 c.c. in Tube 6; 0.4 c.c. in Tube 7; 0.3 c.c. in Tube 8; 0.2 c.c. in Tube 9; 0.1 c.c. in Tube 10; 0.2 c.c. in Tube 11; 0.15 c.c. in Tube 12; 0.1 c.c. in Tube 13; 0.2 c.c. in Tube 14; 0.15 c.c. in Tube 15; 0.1 c.c. in Tube 16; 0.2 c.c. in Tube 17; 0.15 c.c. in Tube 18; 0.1 c.c. in Tube 19; and 0.3 c.c. in Tube 20.

The first ten tubes are used to determine the hemolytic index of the blood under examination. In other words, the object is to find the amount of natural complement and amboceptor in the suspected serum. With Tubes 11 to 19 the actual test is carried out. Tube 20 is the serum control tube. Now add 0.1 c.c. of a 5 per cent suspension of sheep cells to Tube 1; 0.2 c.c. to Tube 2; 0.3 c.c. to Tube 3; 0.4 c.c. to Tube 4; 0.5 c.c. to Tube 5; 0.6 c.c. to Tube 6; 0.7 c.c. to Tube 7; 0.8 c.c. to Tube 8; 0.9 c.c. to Tube 9; and 1.0 c.c. to Tube 10.

Antigen.—Dilute the titrated antigens used in the original technic with equal quantities of normal salt solution.

Place 0.1 c.c. of the diluted Petroff's antigen into Tube 11; 0.15 c.c. of the antigen into Tube 12; and 0.2 c.c. of the antigen into Tube 13.

Place 0.1 c.c. of the diluted Miller's antigen into Tube 14; 0.15 c.c. of the antigen into Tube 15; and 0.2 c.c. of the antigen into Tube 16.

Place 0.1 c.c. of the diluted Fleisher-Ives antigen into Tube 17, 0.15 c.c. of the antigen into Tube 18; and 0.2 c.c. of the antigen into Tube 19.

These nine tubes (11, 12, 13, 14, 15, 16, 17, 18 and 19) are the tubes in which the final readings are made and for that reason the antigen is added to them alone. Tube 20 being the serum control tube, received of course, no antigen. The tubes are now well shaken and placed into the water-bath for one hour at 37.5° C. At the end if this time the rack is removed from the water-bath and the last tube which shows complete hemolysis is the hemolytic index. For example, if Tube 7 is the last where complete hemolysis occurs, the hemolytic index is 7. This means that 0.1 c.c. of serum will hemolyze 0.7 c.c. of a 5 per cent suspension of sheep cells.

In order to determine the quantity of sheep corpuscles to put into Tubes 11 to 20 inclusive, we have used the same table as used in performing the Hecht-Weinberg test for syphilis. If the hemolytic index is from,

1 to 4 add 0.1 c.c. of sheep cells to Tubes 11 to 20 inclusive.
5 to 7 add 0.15 c.c. of sheep cells to Tubes 11 to 20 inclusive.
8 to 10 add 0.2 c.c. of sheep cells to Tubes 11 to 20 inclusive.

After the addition of sheep cells to Tubes 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 the contents of the tubes are well shaken and again placed in the water-bath for one hour at 37.5° C. The rack is then removed and the results read just as in the Wassermann test.

TABLE II

CLINICAL TUBERCULOSIS CASES

	COMPLEMENT-FIXATION TEST FOR TUBERCULOSIS			MODIFICATION FOR TUBERCULOSIS		
CASE NO.	PETROFF ANTIGEN	MILLER ANTIGEN	FLEISHER-IVES ANTIGEN	PETROFF ANTIGEN	MILLER ANTIGEN	FLEISHER-IVES ANTIGEN
8	++	++	++	++++	++++	–
16	++++	–	–	++++	++++	++++
18	++	–	–	++++	++	–
20	++++	++++	++	++++	++++	++++
21	++++	++++	+++	++++	++++	++++
22	+	+	+	++	++	++
45	++++	–	–	++++	–	++++
47	–	–	–	–	–	–
56	–	–	–	–	–	–
57	–	–	–	++	++	+
58	–	–	–	–	–	–

TABLE III

TUBERCULOSIS SUSPECTS

	COMPLEMENT-FIXATION TEST FOR TUBERCULOSIS			MODIFICATION FOR TUBERCULOSIS		
CASE NO.	PETROFF ANTIGEN	MILLER ANTIGEN	FLEISHER-IVES ANTIGEN	PETROFF ANTIGEN	MILLER ANTIGEN	FLEISHER-IVES ANTIGEN
7	–	–	–	–	–	–
11	–	–	–	++	+	+
13	–	–	–	–	–	–
14	–	–	–	++	–	+
26	++	+	+	++++	++++	++++
28	–	–	–	–	–	–
35	–	–	–	–	–	–
38	++++	++++	++++	++++	++++	++++
41	–	–	–	–	–	–
50	–	–	–	–	–	–

TABLE IV

OTHER DIAGNOSES WITH POSITIVE FIXATION TESTS

	COMPLEMENT-FIXATION TEST FOR TUBERCULOSIS			MODIFICATION FOR TUBERCULOSIS		
CASE NO.	PETROFF ANTIGEN	MILLER ANTIGEN	FLEISHER-IVES ANTIGEN	PETROFF ANTIGEN	MILLER ANTIGEN	FLEISHER-IVES ANTIGEN
19	++++	–	++	++++	++++	++++
37	++	++	++	++++	++++	++++
40	–	–	–	++++	++++	++++
42	–	–	–	++++	++++	++++
44	++++	–	–	++++	++++	–
54	++++	++++	++++	NOT		MADE
55	++	+	++	++++	++	++++

Various workers have made different claims as to the value of the complement-fixation test in tuberculosis. Ives and Singer[1] state, "Since the manifestations of tuberculosis are of such protean character that any disease which does not permit a definite diagnosis may be suspected to be of tuberculous origin,[4] and since many tuberculous affections remain unrecognized after the application of all well-tried diagnostic methods, the desirability of a serologic test for tuberculosis, which will be equally as useful in tuberculosis, as is the Wassermann test in syphilis, is evident. It would appear that in the work of Miller and Zinsser[5] such a test has been nearly perfected. Their optimistic views regarding the test as performed by them are shared by Craig,[6] and our work further supports these views."

Pritchard and Roderich[2] conclude, "This reaction does not give as valuable or consistent information in relation to tuberculosis as does the Wassermann reaction in regard to syphilis. In some open advanced cases of pulmonary tuberculosis, no reaction occurred. An explanation of this might be that the cells have lost their power of reaction owing to their prolonged saturation with specific toxins. This test is of greatest help to us in differential diagnosis. It also acts as a stimulus to more careful observation."

Moursund[7] claims that the complement-fixation test as described by him is of no value as a diagnostic or prognostic aid. He also states that not all complement-fixation tests with bacterial antigens are specific and mentions that a large percentage of serums give cross fixation tests in the Wassermann reaction for syphilis and the complement-fixation test for gonorrhea.

CONCLUSIONS

From Table IV it will be observed that in the original complement-fixation test, there were 4.98 per cent positive reactions in diagnoses other than tuberculosis. In the modification there were 9.96 per cent positive reactions, and in the combined tests there were 11.62 per cent. In these cases further studies are being made to eliminate tuberculosis inasmuch as Case 19 had syphilis; Case 37, intestinal toxemia; Case 40, intestinal toxemia with nephritis; Case 42, cholecystitis with syphilis, Case 44, terminal gastric catarrh, Case 54, gastric-coloptosis with chronic appendix; and Case 55, gastrocoloptosis.

Of the ten tuberculosis suspects, there was one positive fixation test in the original technic. In the modification, however, there were two strong positive fixation tests. (See Table III.)

There were 11 cases of clinical tuberculosis, and of these, 4, or 39.6 per cent, were positive in the original technic. In the modification there were 6, or 59.4 per cent, positives. (See Table II.)

From Tables II, III and IV we observe that the modification gives a larger percentage of positive results than the original technic. The writers are fully aware of the fact that the cases here reported are too few in number to draw any definite conclusions, and are therefore continuing this work which will be published later.

ANTIGENS

In the original technic there were eight positive results with Petroff's antigen, 4 with Miller's and 3 with Fleisher-Ives'.

In the modification there were 14 positive reactions with Petroff's antigen, 11 with Miller's and 11 with Fleisher-Ives'.

There was not a single case in which Miller's or Fleisher-Ives' antigens were positive that Petroff's antigen was negative; on the contrary there were cases where the Petroff's antigen was positive and Miller's and Fleisher-Ives' were negative. These conclusions regarding the antigens are not final, as future experiments may prove otherwise.

We agree with Singer and Ives that a serologic test for tuberculosis that will be as useful as the Wassermann test in syphilis is desired, but in our limited experience we observed that the original technic for tuberculosis was not as valuable as the Wassermann test in syphilis. We found that in the 11 cases of clinical tuberculosis 39.6 per cent reacted positive, whereas in the modification there were 59.4 per cent positives. In other words the modification gave 20 per cent more positives than the original test. We will therefore use this modification in conjunction with the original technic with the object of perfecting the complement-fixation test for tuberculosis with the expectation that it may give as valuable and consistent information in tuberculosis as does the Wassermann test in syphilis.

REFERENCES

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[2]Pritchard, J. S., and Roderich, C. E.: Complement-Fixation Test for Tuberculosis, Jour. Am. Med. Assn., Dec., 1919, lxxiii, No. 25.
[3]Blaivas, A. J.: Jour. Lab. and Clin. Med., Jan. 1920, v, No. 4.
[4]Von Ruck, S.: Southern Med. Jour., 1916, ix, 595.
[5]Miller, H. R., and Zinsser, H.: Proc. Soc. Exper. Biol. and Med., 1916, xiii, 134. Miller, H. R.: Jour. Am. Med. Assn., 1916, lxvii, 1519.
[6]Craig, C. F.: Jour. Am. Med. Assn., 1917, lxviii, 773.
[7]Moursund: Jour. Infect. Dis., 1920, xxvi, 85.

LABORATORY METHODS

BODY TEMPERATURE TAKEN IN FRESHLY VOIDED URINE

By Prof. Dr. Theodore Kasparek, Czech University of Prague, Prague, Czechoslovak Republic

IN THE course of investigations on bactericidal agents in freshly voided urine I took also its temperature and found several interesting facts, which led me to further researches in this matter.

For the purpose of determining the exact temperature of urine I have constructed a simple apparatus, consisting of an ordinary glass funnel, with a tube of 7 to 8 inches in length and 3.5 lines in diameter, so arranged as to hold an ordinary, clinical thermometer. The urine is voided directly through the apparatus, which can be placed into an ordinary urinal. In order to prevent loss of body heat, it is previously washed with about 250 c.c. of water, kept several hours in an incubator, or warmed with a properly arranged device of my own construction, at 96° F. This apparatus is manufactured and sold by the Wappler Electric Company, 162-184 Harris Ave., Long Island City, N. Y., under the name of Urothermometer with full directions for its operation.

I have obtained the following average temperatures of normal urine passed through the apparatus:

TIME	TEMPERATURE OF URINES	RECTAL TEMPERATURE	AXILLARY TEMPERATURE
Morning	97.2°-98.0°	98.2°- 98.8°	97.6°-97.6°
Noon	98.6°-99.0°	99.2°- 99.8°	97.8°-98.0°
Evening	98.2°-99.4°	98.4°-100.0°	98.4°-98.8°

In fever cases the difference of temperature between the freshly passed urine (through this apparatus) and the rectal temperature is constant and notable. In a febrile case of Morbus Basedow in which the cause of high temperature could not be recognized at the time—the difference was 0.8° and in a patient with pneumonia the same results were obtained. In several other cases the results, which are presented in Table I, show similar tendencies:

Table I

DISEASED CONDITION	TIME	URINE* TEMPERATURE	RECTAL TEMPERATURE
Meningitis	4:30 P.M.	99.4°	100.0°
Bronchitis	6:00 P.M.	100.6°	101.4°
Tuberculosis	7:00 P.M.	101.2°	102.0°
Pneumonia	4:00 P.M.	101.8°	102.4°
Morbus Basedow	11:00 A.M.	100.2°	101.0°
Pneumonia and Rheumatism	4:30 P.M.	102.2°	103.0°

*Obtained by catheterization.

In the following three cases (Table II) the urinary temperature was higher than the temperature by rectum. This indicates that either the urine temperature depends on some chemical or pathologic processes in the bladder, or at other portions of the urinary tract.

TABLE II

TIME	DISEASE	URINE TEMPERATURE	RECTAL TEMPERATURE
Noon	?	104.0°	98.6°
Noon	?	101.0°	99.0°
Evening	Cystitis	101.2°	99.6°

CONCLUSIONS

The results of this investigation indicate that the simple method of determining the body temperature by observation of the temperature of freshly passed urine is of practical value. This will apply principally to cases in which measurement by rectum is impossible or obnoxious, as, for instance in timid patients (children and hysterics) or in hypochondriacs, who should not be acquainted with the existence of high temperature. It may also be of value as a new diagnostic method in diseases of the urinary tract. This will depend upon a larger number of observations and will be reported in due time.

A LABORATORY CHART FOR RECORDING OBSERVATIONS ON MICROORGANISMS*

By Fred W. Tanner, Urbana, Ill.

ONE of the problems with which the instructor in bacteriology must struggle, is the manner in which the student shall record the observations made in the laboratory. The conclusion which one is quite liable to reach, is that no entirely satisfactory method exists. Each instructor must therefore work out for himself that method which is most closely adapted to the type of and amount of instruction which he may give. The object, of course, is to give the student as much practical and accurate information as possible during the time that he spends in the laboratory to acquire the basic principles of the science.

The laboratory chart described herein is a modification of the Descriptive Chart of the Society of American Bacteriologists and one received several years ago from the Laboratories of Bacteriology of the Michigan Agricultural College. Since it has been of so much assistance to us in presenting the facts of laboratory microbiology and assisting the student to know what data should be recorded, it seemed advisable to describe this chart in the literature. It is hoped that others will do the same thing. There seems to be a lack of this to one who has attempted to find in the literature much material on the teaching of microbiology.

Numerous other charts have been prepared for this purpose. Probably the first attempt at this was the effort of a Committee of the American Public Health

*From the University of Illinois, Urbana, Ill.

Association to systematize the study of bacteria. Charts upon which the various characteristics of bacteria were recorded were filed with their report as published.[1]

BACTERIA

Name of student ____ Desk No. ____

Name of organism ____ Isolated from ____

Method of Isolation ____

Occurrence ____

Importance ____

Arrangement, *single, pairs, chains, fours, cubical packets.* | Size ____

Involution Forms ____

Motility ____ | FLAGELLA No. ..... Attachment, *polar, bipolar, lophotrichiate, peritrichiate.* How Stained .....

VEGETATIVE CELLS, Medium used .....
temp .....
age ..... days.
Form, *spheres, short rods, long rods, filaments, commas, short spirals, long spirals, spindled, cuneate, clavate, curved.*

ENDOSPORES, *present, absent.*
Location of Endospores, *central, polar.*
Form, *spherical, elliptical elongated.*
Limits of Size .....
Size of Majority .....
Wall, *thick, thin.*
Sporangium wall, *adherent, not adherent.*
Germination, *equatorial, oblique, polar, bipolar, by stretching, by absorption of spore wall.*

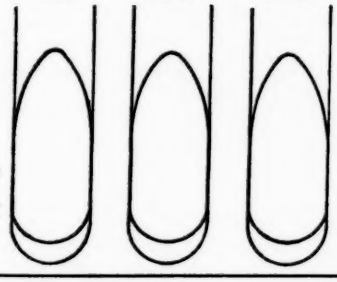
Capsule

STAINING REACTIONS.
1:10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.
Special Stains
Gram .....
Glycogen .....
Fat .....
Acid fast .....
Neisser .....
Metachromatic granules, sporogenous granules.

PLAIN AGAR STREAK

Reaction...
Incubated at
.....°C

....days ....days ....days

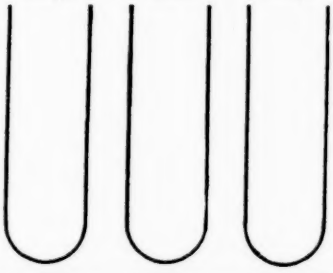


Growth, invisible, scanty, moderate, abundant.
Form of Growth, filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.
Elevation of Growth, flat, effuse, raised, convex.
Lustre, glistening, dull, cretaceous.
Topography, smooth, contoured, rugose, verrucose.
Optical Characters, opaque, translucent, opalescent, iridescent.
Chromogenesis .....
Odor, absent, decided, resembling .....
Consistency, slimy, butyrous, viscid, membranous, coriaceous, brittle.
Medium grayed, browned, reddened, blued, greened.
NOTE.—Underline terms applying to description of cultures.

PLAIN GELATIN STAB

Reaction....
Incubated at
.....°C

....days ....days ....days



Growth uniform, best at top, best at bottom.
Line of Puncture, filiform, beaded, papillate, villous, plumose, arborescent.
Liquefaction, crateriform, napiform, infundibuliform, saccate, stratiform; *begins in* ..... *d, complete in* .....*d.*
Medium fluorescent, browned .....

Page 1 of chart.

[1]Procedures Recommended for the Study of Bacteria with Special Reference to Greater Uniformity in the Description and Differentiation of Species. Being the Report of a Committee of Bacteriologists to the Pollution of Water Supplies of the American Public Health Association, Am. Pub. Health Assn. Proc., 1898, pp. 60-100.

The outgrowth of this early work which has been so well reviewed by Harding[2] is the Descriptive Chart of the Society of American Bacteriologists. This has passed through several revisions and with the Group Number, a numerical method for recording the salient characteristics of microorganisms, represents, probably, the most recent effort on the part of an organized society at

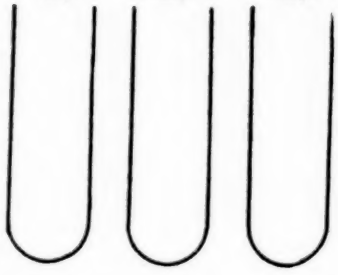
PLAIN BROTH CULTURE

Reaction. . .

Incubated at

..... °C

.days ....days ....days



Surface growth, ring, pellicle, flocculent, membranous, none.

Clouding, slight, moderate, strong; transient, persistent; none; fluid, turbid.

Odor, absent, decided, resembling..... ....

Sediment, compact, flocculent, granular, flaky, viscid on agitation, abundant, scant

Medium, fluorescent..... .....

Age of colony	.....days		.....days		.....days	
Size of colony (Millimeters)						
Surface elevation						
AGAR COLONY	Macroscopic	Microscopic	Macroscopic	Microscopic	Macroscopic	Microscopic
		Edge of Colony Low Power Objective		Edge of Colony Low Power Objective		Edge of Colony Low Power Objective

Reaction....

..... °C

Growth, slow, rapid, temperature.....

Form, punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.

Surface, smooth, rough, concentrically ringed, radiate, striate

Elevation, flat, effuse, raised, convex, pulvinate, umbonate.

Edge, entire, undulate, lobate, erose, lacerate.

Internal structure, amorphous, finely, coarsely-granular, grumose, filamentous, floccose, curled.

Age of colony	.....days		.....days		..... days	
Size of colony (Millimeters)						
Surface elevation						
GELATIN COLONY	Macroscopic	Microscopic	Macroscopic	Microscopic	Macroscopic	Microscopic
		Edge of Colony Low Power Objective		Edge of Colony Low Power Objective		Edge of Colony Low Power Objective

Reaction...

Incubated at

..... .°C

Growth, slow, rapid.

Form, punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.

Elevation, flat, effuse, raised, convex, pulvinate, crateriform (liquefying).

Edge, entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.

Liquefaction, cup, saucer, spreading.

Page 2 of chart.

[2]Harding, H. A.: The Constancy of Certain Physiological Characters in the Classification of Bacteria, Tech. Bull. New York, Ag. Exp. Station, Geneva, 1910.

standardizing the study of bacteria. This chart has been used by various investigators in research work. However, from the teaching standpoint, it has certain disadvantages. The matter which is given on this chart is crowded and there is no room for the various general records which a student must make. No space is left for sketching certain types of growth. Realizing this there was an effort in the society for a number of years to formulate a chart for student use. This idea culminated in the report of the committee on the Chart for Identi-

		..... days	..... days	..... days
Litmus milk	No change			
	Acid			
	Gas			
	Acid curd			
	Rennin curd			
	Reduction			
	Alkali			
	Peptonization			

Fermentation Reactions

% OF GAS IN	CONTROL	DEXTROSE	LACTOSE	SUCROSE	GLYCEROL			
24 hours								
48 hours								
4 days								
7 days								
Total gas production								
Odor								
Acid								
Growth in closed arm								

Production of	NH_3 from peptone	
	H_2S from peptone	
	Nitrites from peptone	
	Indol from peptone	
Reduction of nitrate to	NH_3	
	Nitrites	
Diastasic action on starch	Present	
	Absent	
Chromogenesis on	Nutrient broth	
	Nutrient gelatin	
	Nutrient agar	

Page 3 of chart.

fication of Bacterial Species at the 1917 meeting of the Society of American Bacteriologists wherein was presented a "Descriptive Chart for Use in Bacteriological Instruction." It seems doubtful whether such a committee, very few of whom are actively concerned with teaching laboratory bacteriology, could most clearly realize the needs of the teacher. Those who are giving such instruction regularly and are talking with students through the academic year are probably better qualified to work out such a chart. Various other charts are being

Group Number .....

Special Observation:

Endospores produced 100 Endospores not produced 200.	
Aerobic (Strict) 10. Facultative anaerobic 20 Anaerobic (strict) 30.	
Gelatin liquefied 1. Gelatin not liquefied 2.	
Acid and gas .1 Acid without gas .2 No acid .3 No growth .4 with dextrose.	
Acid and gas .01 Acid without gas .02 No acid .03 No growth .04 with lactose.	
Acid and gas .001 Acid without gas .002 No acid .003 No growth .004 with saccharose.	
Nitrates reduced with gas .0001 Nitrates reduced without gas .0002 Nitrates not reduced .0003	
Fluorescent .00001 Violet .00002 Blue .00003 Green .00004 Yellow .00005 Orange .00006 Red .00007 Brown .00008 Pink .00009 Non-chromogenic .00000.	
Diastasic action on potato starch, strong .000001 Feeble .000002 absent .000003	
Acid and gas .0000001 Acid without gas .0000002 No acid .0000003 No growth .0000004 with glycerin.	

Dec. 31 1914 Chart)

REMARKS

LABORATORIES OF BACTERIOLOGY

University of Illinois

URBANA

May, 1920

Page 4 of chart.

used in other institutions. The Frost Chart is probably well known to most bacteriologists. At Ohio State University and the College of Medicine of the University of Illinois such charts are being used for laboratory instruction. Copies of both of the Descriptive Charts of the Society of American Bacteriologists may be secured from Doctor J. A. Conn, Agricultural Experiment Station, Geneva, New York. The society through its committee has made a commendable effort at providing standard methods for carrying out the observations made on bacteria. Several reports have already been published.

The chart described herein, as stated above, is modified from the Descriptive Chart of the Society of American Bacteriologists. No agreement will exist with regard to what determinations should be made by the student on the various microorganisms which he studies in pure culture. The one described in this paper, we believe, contains these determinations which should be made on organisms in an introductory course in general microbiology. It must be borne in mind that our effort is to give the student the most solid foundation in technic that is possible in the time that he is with us.

It consists of a four-page printed folder 8½ by 11 inches. The student is supposed to use one of these charts for each organism which he studies and to file the charts in his notebook. Spaces are provided for recording both cultural and morphological data. Since the Group Number has been used by the Society of American Bacteriologists and since the laboratory determinations demanded in determining it are those made on microorganisms in almost every introductory course in the science, it has been included. The terms which are underlined in describing the organisms are taken from the Society Chart and a glossary is provided the student at the beginning of the semester. Other features of the chart are the spaces for making sketches. It is well known that difference of opinion exists with regard to the value of sketches. Some regard them as a waste of the student's time and probably, at times, they are very misleading especially if the student attempts to use them after he has progressed further in his course. However, anything which will help to make the student more observing of the differences in types of bacterial development is legitimate.

This chart has some disadvantages like all methods which must be used in presenting the facts of a somewhat inexact science. Copies of it may be secured, if desired, from the writer.

AN IMPROVED METHOD FOR FEEDING RABBITS BY STOMACH SOUND*

By Martha R. Jones, New Haven, Conn.

ALTHOUGH feeding rabbits by stomach sound is a routine procedure in many physiologic laboratories, the device described below was found to be so simple and satisfactory for introducing solutions quantitatively into the stomach that it seemed worth publication.

The rabbit is fastened on an ordinary cat board, the head holder consisting of two upright, perforated pillars through which a horizontal bar can be passed

*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

at any height desired. When handled gently the rabbits, as a rule, do not become excited, and one person can put them on the board with ease. The animal's jaws are then opened with the fingers and a pointed wooden rod about ¾ inch in diameter and 4 inches long with a ⅜ inch hole in the center passing through its short axis is inserted behind the teeth. Burette clamps attached to an iron ring stand are then slipped over the ends of the rod and fastened so that it is fixed in position. The rabbit is now able to open his mouth freely, but is prevented from ejecting the rod from behind his teeth and from biting the sound when it is introduced into the stomach. A funnel to which the sound (small

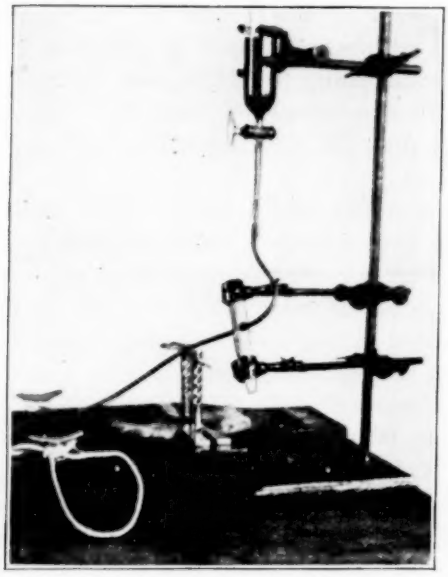


Fig. 1.—Animal board and feeding device.

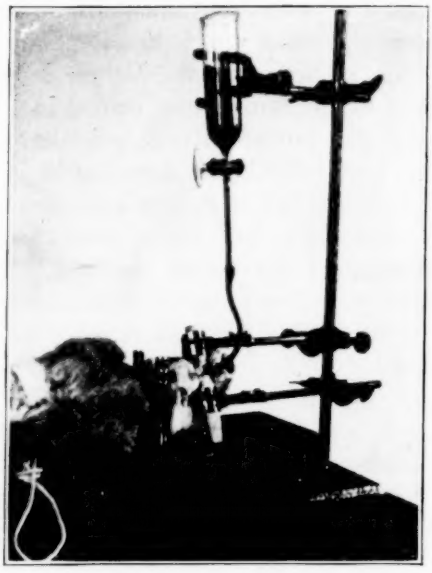


Fig. 2.—Rabbit receiving a dose of sugar.

male catheter) has been attached is supported by another burette clamp. The free end of the tube is now moistened and gently forced into the stomach through the hole in the wooden rod. Usually the sound finds its way into the esophagus without difficulty. Its location, however, can be readily ascertained by gently feeling behind the trachea. The liquid is now poured into the funnel, and if desired, its rate of discharge can be regulated by means of a pinch cock. One great advantage of the device is that with a little practice one person can feed two or more animals at the same time, which is a matter of considerable importance when a large number of rabbits are being fed thick, viscid solutions that pass through the tube very slowly.

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EDITORIALS

Nonspecific Immunity

FROM the struggle between parasitic microorganisms and the forces of the animal body there evolve certain groups of phenomena which we designate by the term "disease." Infectious disease is the result of and the expression of this two-sided combat. In the study of pathological physiology we have been accustomed to observe chiefly the physical and chemical changes produced during manifest disease. But we should not limit our study of the immunologic processes to those produced during acute illness. Manifest disease is only one phase of the constant struggle between invader and host. In nature, the latter overcomes the former many times without the production of clinical disease to each time that it does with such result.

The greater portion of past research work in immunology has been devoted to studies of specific immunity produced against specific antigens. Theories based upon specificity have aided greatly in elucidating our concepts, but time and again, diverse phenomena in disease have occurred which have baffled explanation in the light of those theories.

The relative insusceptibility of city bred over rural recruits in the army even to diseases which they have not previously had is a fact concerning which

there can now be little doubt. There are some who believe in a relative immunity of tuberculous individuals to epidemic influenza. The clinical improvement in certain subacute and chronic diseases, such as lupus vulgaris, after the occurrence of a superimposed acute infection, as erysipelas, is a matter of common knowledge. We observe that one of the acute exanthemata rarely if ever occurs coincidently with another. Such forms of insusceptibility appear to be nonspecific in character, and it is because of these and similar phenomena that the existence of a more or less general nonspecific protective mechanism has been suggested.

That there does exist, in addition to natural and acquired specific immunity, an acquired nonspecific immune mechanism seems most probable. It is perhaps never of as great degree as the other two forms, but often confers enough protection to be the deciding factor between life and death of the individual. Thus, W. T. Vaughan and Schnabel found that among ninety soldiers with bronchopneumonia who had had no previous history of specific infections, the mortality was 47 per cent, while among 243 who had averaged nearly two previous infections apiece, the death rate was under 35 per cent. The general mortality among 234 cases of lobar pneumonia was 14.9 per cent, while of ten cases with entirely negative past histories for infectious disease, 20 per cent died. The individual who had had other infections previously, stood a better chance of winning than the one who had never been called on to fight a battle.

V. C. Vaughan and Palmer called attention to the fact that the seasoned soldier is more resistant even to newly imported infections than the recent recruit. They showed that in the army camps in 1917-1919 the men from crowded cities resisted the respiratory infections more successfully than their comrades from sparsely settled areas.

Later, Love and Davenport also showed that the communicable diseases produced the least admission and death rate in those camps which drew from areas that were prevailingly urban; that the highest morbidity and mortality rates were from camps that drew from the sparsely populated areas of the Southern Atlantic and Gulf States, and to a less extent from the sparsely settled states of the west. They demonstrated these facts mathematically by the method of multiple correlation. The results with both groups of investigators were the same not only for measles and mumps, diseases which the urban recruits had probably had in childhood, but also for pneumonia, cerebrospinal meningitis, influenza and scarlet fever, from which the majority had probably never suffered. This last fact negatives the theory that urban recruits were less susceptible to the specific infections because they had previously had them. Such an hypothesis holds good for measles and mumps, but not in general. Another explanation, the theory of the elimination of susceptibles in childhood, has much to commend it, but it only carries the solution one step farther. Why are those that remain not susceptible? Again we find ourselves face to face with nonspecific immunity.

In the study of epidemics it is often observed that the year following an epidemic of measles in a large city is one of low mortality, not only for measles, but also for whooping cough and scarlet fever.

The mechanism of nonspecific immunity is undoubtedly complex. Presumably it works on the same general principles as in specific immunity. It may be

conceived of as a grade between the latter and natural immunity, having features common to both types but more closely allied to the acquired variety. Many of the processes in natural immunity are nonspecific. The intact skin and mucous membranes form the first nonspecific barrier. The physical or chemical qualities of the salivary, gastric and intestinal secretions play a part. After microorganisms have penetrated the skin the local leucocytosis combats the infection. Phagocytosis, special tissue insusceptibility, ferments or antibodies, antitoxins, bacteriolysins and precipitins all play a part in natural as well as acquired immunity and these must all be investigated in a study of nonspecific immunity.

It may be found that there are even new factors in this form of protection. According to Wooley, 3, 757 of 9,559 men who had been in camp less than five months developed influenza in the autumn, 1918, epidemic at Camp Devens, while only 1,033 of 5,943 who had seen more than five months service took sick. At Camp Lee, Lt. Wallis reports that those who had been in the service less than one month constituted only 9 per cent of the total strength, but furnished 30 per cent of the total influenza deaths; that those who had been in service over three months constituted 45 per cent of the camp population, but furnished only 23 per cent of the deaths. At Camp Custer 64 per cent of the men who had influenza had been in the service only two months.

Length of service evidently had some bearing on influenza immunity. One may explain that the older soldiers had become acclimated to their surroundings, but the fact remains beyond this, that the older soldier, no more *naturally* immune than his more recent associate, was less susceptible to the ravages of the disease, and that this relative insusceptibility was in no way specific in character. So, physical condition or metabolic equilibrium may be an additional factor to be studied in nonspecific immunity. Indeed this is a factor of other forms of immunity. Pasteur demonstrated years ago that the natural immunity of birds to anthrax could be overcome by exposure and fatigue.

Vaughan and Palmer suggest an explanation of the mechanism of nonspecific immunity which is in harmony with existing theories. The bacterial protein, when it is digested in the body is broken down into two portions; one specific, different for each different protein; and not poisonous; and the other, nonspecific, alike in all forms of protein molecules, and poisonous. By injection of the specific fraction of the bacterial molecule a true immunity can be produced against the living germ, which will protect against from two to twelve times the minimum lethal dose of that germ. It will not protect against any other. Thus is specific immunity developed by the liberation in the body of this specific antigenic fraction during destruction of the germ. The poisonous portion when injected produces no such immunity but does result in increased tolerance towards itself on the part of the body, so that sometimes as much as twice the minimum fatal dose may be injected without fatal result. It is this poisonous portion of the molecule which during destruction of the germ within the body produces the general symptoms of disease. It is the same whatever be the organism causing the disease.

So, one who has *recovered* from measles has acquired through the action of the nonpoisonous fraction of the measles virus protein, a specific immunity of high potency, and, at the same time, an increased tolerance to the poisonous frac-

tion, nonspecific, and of low potency. Subsequent infection, say with small pox virus produces a mild attack because of the lessened susceptibility to the poison.

Here is a theory of nonspecific immunity which has the advantage of being a good working hypothesis and of being in harmony with existing theories. That theory to which new developments in immunology can be most successfully and most consistently applied will eventually be found to be the truest explanation of the mechanism of immunity.

One apparent drawback to the hypothesis is that the increased tolerance induced by the protein poison is, experimentally, of short duration, while nonspecific immunity appears to last for months or years. Further investigation will help to solve this problem.

Another explanation derived from the theories of Jobling on nonspecific protein therapy deserves mention. In it the nonspecific element is a proteolytic ferment mobilized in the blood following experimental inoculation of bacteria. This ferment has no action on bacteria, but he suggests that "if we consider the source of the intoxication as primarily due to protein split products, then a mobilization of protease may be of considerable importance in the process of detoxication, in that the toxic fragments are hydrolyzed to lower and nontoxic forms." Thus it is suggested that the nonspecific element is the ferment that helps to destroy the bacterial cell.

It is doubtful whether this form of immunity can prevent infection with a newly imported virus. But that it can mitigate the effects of the new infection seems quite probable.

The subject of local tissue immunity is difficult to study experimentally and has in the past usually been neglected for the investigation of general immunologic processes. There is some evidence pointing to local nonspecific protection. Armstrong believes that the chronic pulmonary infection with the tubercle bacillus and its accompanying secondary invaders rendered the tuberculous less susceptible to the acute respiratory infection of influenza. The Typhoid Commission in the Spanish American War concluded that temporary gastrointestinal disturbances instead of predisposing to typhoid fever, gave a certain degree of immunity against subsequent infection with this disease.

Nonspecific immunity, if such does exist, represents a radical departure from present day concepts in which specificity is the keynote. Clinical data such as those already quoted are abundant, but are so general in character that they may be misleading. Experimental evidence must be clear cut and convincing.

If the theory is corroborated many phenomena hitherto unexplained will be understandable. Much, who has written at some length in the *Deutsche Medizinische Wochenschrift*, attempts to explain thereby, results of vaccination against migraine, the use of microorganisms in the treatment of cancer, shot gun vaccines in various maladies, autoserotherapy in skin affections, high mortality among the robust in pandemics, and many other conditions. While these things may be in some manner related, one must be cautious not to endeavor to explain too great a variety of phenomena before he has proved satisfactorily that nonspecific immunity exists and how it operates.

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—*W. T. V.*

Recent Researches on the Capillary Circulation

IT HAS been the custom to assume that the walls of the capillaries are incapable of constricting or dilating independently of changes of pressure in the blood circulating in them. According to this view the magnitude of the capillary circulation depends on the amount and pressure of the blood which enters the capillaries from the arterioles and it cannot become altered by local changes in the capillaries themselves. A. Krogh has, however, brought forward unassailable evidence to show that this view is in general incorrect. He has shown that the capillaries possess independent powers of constricting and dilating, and also that they are very much less in caliber when the tissue which they supply is at rest than when it is active.

The observations from which the conclusions are drawn have been published in two papers,[2, 3] those of the first being made on the vessels of muscles, in frogs and guinea pigs, and those of the second on the vessels of the tongue in frogs. The method most frequently employed to render the vessels visible was examination with the binocular microscope by reflected light, although much information was secured by injecting intravenously a solution of India-ink, then killing the animals and examining either fresh or fixed tissues by ordinary methods of microscopy to determine the capillaries into which the black particles have penetrated.

In resting muscle relatively few capillaries were visible, these being, however, evenly distributed to form an elongated mesh work along the muscle fibers. In active muscle, on the other hand, many previously invisible capillaries came into view, to disappear again when the contraction was over. These differences could even be made out macroscopically, after injecting India-ink by the almost black color of muscles that had been active when the animal was killed in contrast with their pale gray appearance when at rest. An observation of great interest is that the corpuscles often crowd themselves through capillaries having diameters which are much less than those of the corpuscles. In order that they may pass, the corpuscles become folded or sausage shaped and the capillaries become deformed, showing marked bulgings over the corpuscles. The great variability in the number of patulous capillaries, according to the state of activ-

ity of the tissues, indicates that alterations in oxygen supply, to meet the varying demands is probably accomplished by greater blood flow. This possibility is considered in another paper[1] in which, by mathematical calculation based on (1) the depth of tissue which each capillary supplies; (2) the rate of oxygen consumption by the tissue, and (3) the diffusion rate of oxygen, it is shown that the oxygen pressure necessary to supply the muscle fibres is so small, even during their greatest activity, that the call for oxygen can readily be met by diffusion alone. This conclusion is in conformity with the facts that the partial pressure of oxygen in the urine is the same as that in venous blood and that when a neutral gas, such as nitrogen, is placed in the pleural or peritoneal cavities it ultimately becomes mixed with oxygen up to a percentage of three or four. That no oxygen should be detectable in tissues such as muscle is explained by its immediate utilization for purposes of metabolism.

Further light has been thrown on the nature of the capillariomotor mechanism by examination through the microscope of the blood vessels, particularly of the frog's tongue, during either mechanical irritation, or the action of certain drugs. In reporting the far-reaching results which he has succeeded in obtaining on this aspect of the problem, Krogh points out that the capillaries of various tissues, just like the arterioles, differ considerably in their reactions. The exact nature of the capillariomotor mechanism must therefore be investigated for each tissue separately and general conclusions applicable to all vascular areas cannot be drawn.

When the surface of the ventral aspect of the tongue (of the frog) is scratched with a fine glass pencil, or with a hair, it can be seen, after a latent period of 10 to 15 seconds, that both the capillaries and the arterioles dilate over an area which is greater than that stimulated. The venules, however, do not show any change apart from that which can be accounted for as secondary to changes in the amount of blood flowing into them. Taken alone, the results might indicate that dilatation, or constriction, of the arterioles merely causes more, or less, blood to flow into the capillaries. That another factor is also involved, namely, independent changes in the caliber of the capillaries, was shown by the following experiment: (1) After greatly slowing down the blood flow and reducing its pressure on one side of the tongue, by partially clamping the corresponding lingual artery, mechanical irritation had its usual effect in dilating the capillaries, the blood flowed slowly into them and in place of the corpuscles having to pass as sausage-shaped bodies one by one, several could now pass side by side. When the clamp was removed no further dilatation of the capillaries occurred, although the blood current became much more rapid and several capillaries which previously had not been visible sprang into view. Of course, when the artery was completely occluded, irritation did not cause any visible change in the capillaries, but when the block was subsequently removed the arterioles and capillaries immediately became filled with blood in a dilated state, showing that the irritation produces a relaxation of the walls, but that some blood pressure is necessary to bring about an actual dilatation. (2) Sometimes it was possible to localize the stimulation so that capillaries became dilated without any change in the arterioles. In such cases the blood flow in the capillaries might actually stagnate and the corpuscles either stand still or move backwards from

the venous end toward the arterial. In one case a local dilatation was noticed to occur at the arterial end of a capillary, and the venous end remaining constricted, the blood flowed into the dilated portion, but stasis occurred. The venous end of the capillary was then irritated when it opened up and the blood current through the whole capillary was reestablished. In another case a very long capillary was caused to dilate at two separate places, by mechanical stimulation, and at these places the corpuscles moved with extreme slowness, although moving quickly in the original capillary. In yet other cases the venous end of a capillary, perhaps previously invisible, could be caused, by irritation, to open up so that blood flowed back into it from the venule and, by repeated repetition of weak stimuli in front of the visible column of blood, the filling could be extended backwards until another vessel with higher pressure was reached when a current became set up in the whole length of capillary. This observation is particularly important in that it shows the state of the capillary wall to be much more important in determining the blood flow through them than the blood pressure in the arterioles. (3) Important reactions were observed to occur towards certain chemical substances. Methane (25 per cent solution) caused the capillaries to become enormously dilated and to assume a varicose appearance, while the arterioles remained unaffected. When the supplying arteriole was of narrow caliber, the blood when it enters the dilated capillary, might stagnate completely so that even after several days it was filled with a stationary mass of corpuscles. Iodine, in 1 per cent solution, caused a similar capillary dilatation, but the arteries were likewise involved, although to a less degree. The muscles of the tongue also contracted strongly when iodine was applied.

Besides the direct evidence which is furnished by the above observations much of a less direct nature was collected to show that the capillariomotor and arteriomotor mechanisms are distinct and separate. For example, pricking the wall of a small artery with a fine hair might cause this vessel to constrict while the capillaries showed the usual dilatation. Electrical stimulation did not yield satisfactory results, but local warming caused dilatation both of arteries and capillaries, and marked hyperemia. Weak acids (1 per cent acetic of pure CO_2) had an effect like that of warming. Adrenin (1 per cent adrenaline), cocaine (2 to 5 per cent), nicotine (0.5 per cent) and amylnitrite all caused the capillaries, and usually the arteries, to dilate. In certain cases, however, it was observed that adrenin caused constriction upon a few small arteries.

One of the most important questions which the above observations raise is with regard to the mechanism involved in causing the changes in the vessels. Is the effect a direct one or is it reflex, and mediated through the central nervous system? Krogh employed two methods to study this question. In one of these the tissue was bathed in a solution of cocaine that was not of sufficient strength to cause marked dilatation of the vessels. In from 30 to 60 minutes the cocaine paralyzed the local nerve endings, with the result that neither mechanical stimulation nor 1 per cent iodine could any longer bring about dilatation, and methane had much less effect than the usual. The other method consisted in observing the effects of local stimulation after cutting the nerves to the tongue (hypoglossal and glossopharyngeal) and allowing them to degenerate. Immediately after section there was often a distinct hyperemia of the corresponding side of the

tongue, but local irritation was still followed by the usual response on the vessels, although this was less evident because of the hyperemia. It is considered likely that the hyperemia following cutting is due to mechanical irritation of the nerve and not to the removal of tonic constrictor impulses. Local freezing of the nerve was, for example, not found to lead to hyperemia, and freezing, of course, blocks the passage of nerve impulses. After time had been allowed for degeneration to occur (eight days) the vessels in the area supplied by the nerve reacted to stimulation much more locally than usual, that is, the effect did not spread much beyond the actual point that had been stimulated. The characteristic effect of the nerve degeneration was, therefore, sometimes a weakening and, always, a marked localization in the response of the blood vessels to mechanical and chemical stimulation.

The conclusions that are drawn from these researches are that there are nerve endings on the arterioles and capillaries and that these have a double function: the one sensory—particularly toward mechanical stimuli—and the other dilatory—that is, inhibiting the vascular tone. Some of the (sensory) endings also exist in the intervascular spaces. When the endings are stimulated the impulse travels up the branch of the nerve fiber with which the ending is connected, and when it arrives at the place of branching the impulse travels down the nerve fiber and is carried by certain of the remaining branches to the arterioles and capillaries, which it causes to dilate. The mechanism is therefore of the type known as an axon reflex. This conclusion, it will be noted, is in harmony with that adopted by Ninian Bruce[4] to explain the results of his observations on the effects which cocainization or nerve degeneration have on the vascular reactions following irritation of the cornea. They also conform with the view of Bayliss[5] that vasodilator impulses are transmitted to the blood vessels of the extremities along sensory nerves.

As a result of nerve degeneration or cocainization the endings are paralyzed, but the vessels (arterioles and capillaries) still maintain their tone and this can still be inhibited by certain forms of stimulation, particularly by methane and weak acids. This indicates that the tone does not depend on nerve impulses, the function of these being solely to inhibit, (or augment) the tone, and to mediate the spread of any change in caliber over a greater stretch of vessel. The tone, therefore, depends on a chemical action of the blood itself and when the supply of blood is cut off for some time the vessel becomes dilated, as is evidenced by the great blood flow which occurs through it when the obstruction is removed. These observations call to mind the conclusions drawn by H. H. Dale and A. N. Richards,[6] and Dale and Laidlaw,[7] concerning the locus of action of histamine and also those concerning the pathogenesis of surgical shock. They are of fundamental importance in the interpretation of many other of the problems that confront the research worker, and with the conclusions which have been drawn from them must be harmonized our views of the pathogenesis of inflammation, lymph formation and the effect of toxic substances.

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—*J. J. R. M.*

Universal Immunity to Tuberculosis

IN a recent issue of this JOURNAL a brief account was given of an experiment in preventive medicine in which a large population is serving to show that thyroid abnormalities may be greatly diminished by a simple procedure. At the same time less circumscribed work on other preventable diseases is being carried on in all parts of the world by the International Health Board and by the League of Red Cross Societies. Uncinariasis, typhus, yellow fever, malaria and plague are subjects for particular attacks. Typhoid fever is for the most part under control, and the method of control is known and is generally available. The great plagues are being fought to the death. The nations of the earth are joining hands to wipe out pestilences.

During the years of organization against disease, tuberculosis has received some large attention to be sure, but the emphasis has been placed largely upon cure, less upon prevention. Experiments in immunity have been carried on, but always under a handicap. Everywhere in the so-called civilized world the disease is endemic and therefore the possibility exists that the experiments are subject to chances of contamination from an infected environment. Notwithstanding the difficulties certain very definite data have been accumulated, and these Calmette states briefly as follows:

Infection with tubercle bacilli, so wide-spread among all civilized nations, is rare, or does not exist among savage races or among the wandering tribes in those parts of the globe which are still isolated from the great commercial routes. It is a disease of crowded social communities, its prevalence and gravity increasing with the density of the population.

It is the same with susceptible animals such as cattle. In the wild states they are free from this disease, whereas the domesticated species, obliged to live in contact with man, are affected in proportion to the closeness of the contact. In towns and in overcrowded stables, in the presence of animals or men who are ill or who, even if apparently healthy, are sowers, as it were, of bacilli, this contagion is almost inevitable. The infection is shown to have already been acquired at an early age by a great number of individuals, and the judicious use of tuberculin reactions has taught us that in New York, just as in Paris, Vienna, Berlin, or London, 55 per cent of the children who have reached their fifth year, and more than 95 per cent of adults, harbor the tubercle bacilli. This does not, however, mean that all these persons are tuberculous.

The disease tuberculosis is one thing; mere bacillary infection is another. The bacillary infection may remain occult, that is to say, it may for many years, or even during the lifetime of the subject, produce no tuberculous lesion; or it may cause a benign tuberculous lesion, liable to remain indefinitely latent. The disease tuberculosis is the result either of a single massive bacillary infection, or of frequent and copious reinfections.

Massive bacillary infection in a subject hitherto free, a young child for instance, or in adults belonging to countries free from tuberculosis, natives of Central Africa, produces severe manifestations which, developing rapidly, are almost always fatal and of various types, according to the number of the infecting bacilli, their origin, and the organs attacked. Massive infection or frequent and copious reinfection supervening on a pre-existing occult bacillary infection or a latent lesion, gives rise to resistant types of disease. The gravity of these types is in close relation to the age of the original infection and to the number and source, and hence the virulence, of the reinfecting bacilli, and also to the seat of the lesions and the anatomical relations of the organs affected.

Experiments on susceptible animals and clinical observations on human beings have justified the assertion that a mild infection occurring in childhood and remaining occult or latent for several years, endows the organism with a pronounced resistance to subsequent inoculations or reinfections. Any individual with an occult bacillary infection or with a benign and latent glandular tuberculous lesion, who is protected, either by his mode of life or by suitable prophylactic measures, from massive or frequent contaminations will retain for years, as long as he reacts positively to tuberculin, this special immunity, which is manifested by a cellular intolerance towards reinfecting bacilli.

It must, therefore, be admitted that acquired immunity to tuberculosis exists. Moreover it is known that it may be conferred artificially on susceptible animals at an early age by careful introduction into their lymphatic organs of small quantities of bacilli whose virulence has been diminished or modified so that they are no longer capable of producing tubercles. It persists as long as the symbiosis of the attenuated or modified bacilli with the lymphatic cellular elements is maintained. It ceases when they are completely eliminated or destroyed and when sensitiveness to tuberculin has disappeared.

These scientific facts show that active immunization against tuberculosis is possible. The question is to find a procedure which will be effective and harmless.

It is, as Calmette says, impossible to carry out the necessary experiments in a civilized community, because of the general environmental infection, a state of affairs which is too apt to modify or make valueless the studies. He says that the necessary conditions can be found only in aboriginal districts in which tuberculosis is nonexistent, and in which native animals can be used for experiment. He suggests an island in French Guinea where chimpanzees can be obtained and cared for by carefully selected healthy natives. These animals could be used not only for experiments in tuberculosis but also for other problems in biology and experimental psychology. Surely the opportunities offered by such an experiment station would be great. The possibilities from the standpoint of general biology are of almost inconceivable magnitude.

It is to be hoped that the plan can be adopted, and financed, and controlled under the supervision of international learned societies.

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—*P. G. W.*

Cancer Among Dye Workers

WE ARE as yet quite ignorant of the actual cause or causes of tumor growth. It may be that it depends upon a parasite, or, because of the multiplicity of tumors, upon a number of parasites. It may be that the tumor cells because of some fundamental biologic change become parasitic—are themselves parasites—and, acquiring independence of their normal environment, go forth into the wider fields of the body, become free lances, marauders, thieves and assassins. Behind such a change may be some extrinsic chemical, or physico-chemical process encouraging the anarchism. It may be true that tumors are as a rule due to a chronic irritation of a chemical nature just as, it seems, they sometimes arise because of chronic physical ones. But even so is the difference between the two processes so fundamental? One may only guess, but in so doing one might conceive that a mechanical influence acting over a certain period of time could have the same effect as a dilute chemical substance—a toxin for example. One might ask whether the "paraffin cancers" of the skin are due to the paraffin acting as a local stimulant, or whether they are an expression of long-continued mechanical stimulation permitted by the lubricant, and therefore soothing, action of the substance. Perhaps without the paraffin the irritation would lead to protective and curative measures, and therefore to prevention.

Certainly there are circumstances which point very directly to a mechanical cause for some tumors. Just as certainly there are more circumstances which point to a chemical cause. In many other cases the two cannot be separated—and this is particularly true in cancers of the gastrointestinal tract in which the growths predominate at the points where trauma is most possible, and where chemical substances formed by bacteria and by the tissues have most chances to act.

In America, where the dye industry is being established widely, we are confronted by the possibility that hitherto uncommon forms of cancer will appear, and many at least of these seem to be attributed to chemical stimulation. In this group are the urinary bladder growths which form the basis of important recent reports.

The dye workers' cancers are interesting because of the fact that they are apparently of pure chemical origin, and because they are of very long incubation. They seem to be due, in the case of the bladder tumors, to the long-continued action of a dilute material, excreted by way of the urinary tract, which affects only the mucous membrane of the urinary bladder. This irritating substance, or the ones from which it originates, may be taken into the body through the skin, the respiratory tract and the gastrointestinal tract, and is generally supposed to be aniline or fenzidine. Nassauer believes that the former alone is the specific one.

Despite the long incubation period Nassauer thinks that workmen should not be exposed for more than three months. He also believes that by enforcement of proper rules of sanitation including personal cleanliness most of the danger can be avoided. In a factory which he has studied, in which many cases formerly occurred, no case has appeared in a period of fourteen years since the

inauguration of strict hygienic requirements. It will be interesting to watch the development of studies made in dye works in America. It will be still more interesting if by taking proper care the appearance of tumors can be eliminated in our dye workers.

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—*P. G. W.*

Pellagra Among Turkish and German Prisoners of War in Egypt

BIGLAND[1] and Enright[2] report small epidemics of pellagra among Turkish and German prisoners of war in Egypt during 1916-1918. The former made a careful study of this disease in sixty-four cases. These were distributed among nationalities as follows: Turks, forty-six; Arabs, eight; Kurds, five; unknown, five. An attempt was made to determine the percentage of pellagrins among the Turkish prisoners. Bigland examined 3,823 in one camp, where he found twenty-four certainly pellagrins, and twenty-one doubtful. At another camp he examined 100 cases without finding any signs of pellagra. In still another place, he studied 470 Turkish women and children without finding any evidence of this disease. Among 4,400 cases he found sixty-four pellagrins, making a percentage of about 1.4. Inasmuch as pellagra is endemic in Egypt, Asia Minor, and Turkey in Asia and Europe, Bigland attempted to ascertain how many of these cases were chronic pellagrins. He concluded that the majority of them were suffering from the first attack.

Arsenic and adrenalin were tried as curative measures but in no instance was any apparent change secured thereby. The only thing which seemed to benefit the patients was a more liberal and varied diet. The most important point discussed by Bigland is as to food deficiency causing the disease among the Turks. He concludes that both before and for a time after capture these men had an insufficiency of food. He believes that this was the cause of the disease in most but not in all of the cases. In order to account for the appearance of pellagra among those sufficiently well fed Bigland resorts to the toxin hypothesis. He says:

"This brings me to the second supposition regarding the etiology of pellagra —viz., the presence of some toxic substance. I would suggest that such a toxin is present in these cases, and that it acts upon the intestines or the contents thereof in such a way that protein is not assimilated as it ought to be. * * * It only remains to discuss what is the nature of this hypothetical toxic substance. It must enter the body from without. It cannot be the result of a metabolic error, because the disease occurs in epidemics. It is difficult to imagine, for example, an epidemic of diabetes. It cannot be carried by the bite of an insect for reasons already stated. It has nothing to do with malaria or dysentery because pellagra

[1]The Lancet, London, May 11, 1920.
[2]The Lancet, London, May 8, 1920.

was unknown in our troops who suffered from both diseases. It may be contained in ingested material, but, if it is, it must be something common to the whole world since cases occur in such different localities. Its probable habitat is the digestive tract, as evidenced by the gastrointestinal symptoms which usually appear first, and the indicanuria, and by the fact that protein is found in the stools in abnormal amounts. It may be, however, that this toxic substance makes its effect by attacking the endocrine organs, especially the suprarenals. The similarities of pellagra to Addison's disease are too obvious to detail. The toxin is apparently of the nature of a virus, since the nervous changes show unmistakable evidence of degeneration rather than of inflammation. That this substance is not transmissible is shown by two facts: (1) That no attendant on pellagrins has ever contracted the disease; (2) the heroic experiments of Goldberger, in which feces, urine, scales from the skin, and all manner of horrible matters arising from pellagrins were eaten by volunteers with no evil results, even after injections and inoculations on mucous surfaces."

Blood cultures and bacteriologic studies of the feces, supplemented by inoculations of animals, were without results. It will be seen that on the whole Bigland confirms Goldberger's theories. He holds, with Wilson, that, although vegetable and meat proteins may be similar, they differ markedly in their nutritive value largely because the former is not assimilated so readily and so completely as the latter.

Enright studied sixty-five cases among German prisoners and comes to the conclusion that his observations are that the "food-deficiency theory" is inadequate. He says:

"I do not mean to suggest that a food deficiency can be quite excluded, but from the facts before us it can hardly be considered as of paramount importance. Obviously something more than a dietetic factor is involved. A critical analysis of the diets which these German pellagrins ate before capture and during their period of captivity prior to the onset of the eruption is sufficient to explode the food-deficiency theory as a predisposing factor. These diets were ample, both in quantity and quality, for any possible requirements, and were of such varied composition that it is perplexing to understand how they could have been improved. * * * Let us consider how the beneficial effects of a diet sufficient for a normal healthy individual, doing a normal amount of work, are liable to be counteracted. An originally good diet may be vitiated by loss through (1) abnormal expenditures of energy, (2) defective assimilation consequent on impaired digestive function, (3) absence of some internal secretion which is necessary for protein metabolism. * * * That the pellagrins suffered from defective digestive assimilation is undoubted. The incidence of malaria and dysentery was very high, and was responsible for intestinal derangement, which was a marked feature of the majority of my cases. Judging from the indicanuria commonly present and the copious foul-smelling evacuations which frequently contained undigested food material, it is evident that there was abnormal protein decomposition and that the ultimate good effects of the diet were largely vitiated or entirely lost in this matter."

Enright found among the German pellagrins a number with parotitis and he raises the question of whether the parotid gland has any effect upon protein metabolism. This is a question which he admits no one can at present answer.

—*V. C. V.*

An Attenuated Tubercle Vaccine

RAW,[1] the distinguished tuberculosis specialist at Liverpool, thinks that human and bovine tuberculosis are separate and distinct diseases, but that the human body is susceptible to both, especially to bovine tuberculosis in the early periods of life. The two diseases are so rarely seen in the same subject that there are strong grounds for presuming that they are antagonistic to each other and that bovine tuberculosis may confer an immunity against human tuberculosis and vice versa.

Possessed of this idea Raw has secured nonvirulent cultures of both human and bovine bacilli. From these he prepares separate tuberculins. Cases of glandular tuberculosis, and all tuberculosis in children, which is evidently of bovine origin, he treats with the tuberculin made from the human type, while, on the other hand, cases of pulmonary tuberculosis, due to the human type, he treats with tuberculin made from the bovine type. He says that tuberculin should always be prepared from attenuated and nonvirulent cultures; that it should be freshly prepared and used within a week; that it should be given in graduated and increasing doses at intervals of seven days; that acute reactions are not necessary; that not less than twelve injections should be given at intervals of one week, and that the most favorable cases for treament are local lesions, but early cases of pulmonary tuberculosis may be limited and a further spread to other parts of the lungs prevented.

—*V. C. V.*

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[1]British Medical Journal, April 17, 1920.

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